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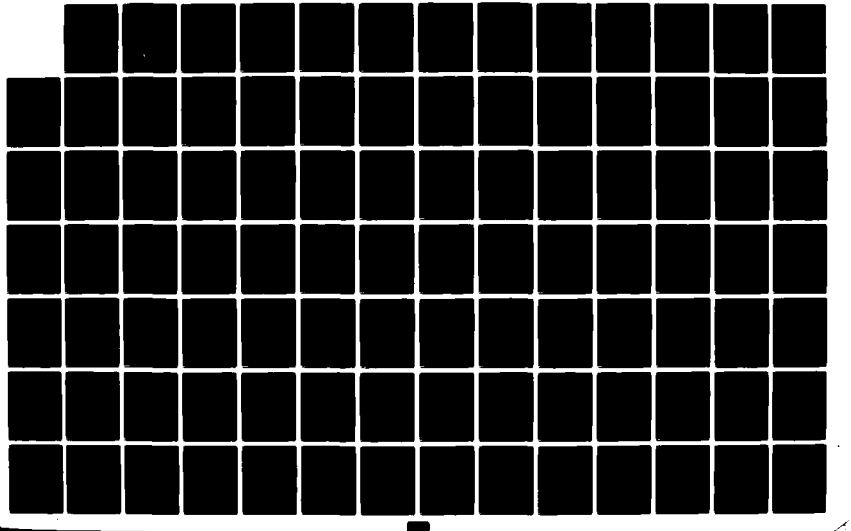

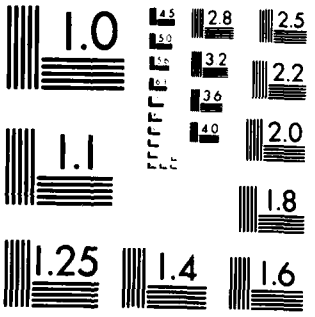


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# BIOTECHNOLOGY: THE FORGING OF MULTIDISCIPLINARY STRATEGIES FOR RESEARCH IN BIOMOLECULAR ELECTRONICS, MATERIALS SCIENCES AND MICROECOLOGY

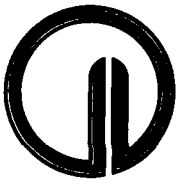
A REPORT ON THREE WORKSHOPS

CONDUCTED BY THE

NORTH CAROLINA BIOTECHNOLOGY CENTER

IN COOPERATION WITH

UNIVERSITY SCIENTISTS AND ENGINEERS



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| Biomolecular Electronics | Materials Sciences | |
| Genetic Engineering | Microecology | |
| Interfaces and Surface Properties | Molecular Switching and Storage | |
| Macromolecular Synthesis | Scale-up | |

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The North Carolina Biotechnology Center, in cooperation with university scientists and with support from the Office of Naval Research, conducted a series of three workshops on the general theme, "Biotechnology: The Forging of Multidisciplinary Strategies for Research." The workshops

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20. ABSTRACT (Continued)

were designed to cover areas of common interest to the Navy and North Carolina scientists and engineers. Each workshop explored the opportunities presented by the merging of biotechnology into a particular area; these three areas were (1) Biomolecular Electronics, (2) Materials Sciences, and (3) Microecology.

The workshops were an experiment -- an experiment in convening scientists and engineers from diverse backgrounds to examine new areas of science and technology and formulate concrete recommendations for future research, and an experiment in the effectiveness of a new state-based agency, the North Carolina Biotechnology Center, in working with university scientists and engineers in planning and conducting such a multidisciplinary process. The results are most encouraging and point the way to considering how to refine and strengthen these organizational and institutional innovations so as to encourage continued collaboration in carrying out the research recommended.

The success of the workshops was due in large part to the nature of the interactions among all those involved. No single individual, group, or organization controlled the action. Rather the key functions were shared among all the members of the Planning Committee and their institutions. Maintaining such a mode of operation over the long term will be essential to any multidisciplinary, multi-institutional group that aspires to pursue the research areas recommended in this report.

Each workshop group produced a set of recommendations for future research. The recommendations are presented by time frame (near term, middle term, long term) and by general subject category.

# BIOTECHNOLOGY:

# THE FORGING OF MULTIDISCIPLINARY STRATEGIES FOR RESEARCH

# in

# BIOMOLECULAR ELECTRONICS,

# MATERIALS SCIENCES AND

# MICROECOLOGY

A Report on Three Workshops
Conducted by the
North Carolina Biotechnology Center
in Cooperation with
University Scientists and Engineers

North Carolina Biotechnology Center
Post Office Box 12235
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December, 1982

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## EXECUTIVE SUMMARY

Biotechnology or biomolecular engineering is an evolving field of immense and diversified potential. Those looking ahead to the future of technological innovation have perceived that integration of recent findings in molecular biology with a variety of other lines of research can lead ultimately to important breakthroughs. The challenge is to achieve this integration. How can scientists who differ in expertise and professional perspectives communicate with each other sufficiently to forge the multidisciplinary research projects necessary for the potential of biotechnology to be fulfilled?

The North Carolina Biotechnology Center, established by Governor James B. Hunt, Jr. in November, 1981, is designed to encourage and facilitate interactions among research scientists, universities, industry, venture capital groups, and government in the development and application of biotechnology within the state. In accordance with these objectives, the North Carolina Biotechnology Center, in cooperation with university scientists and with support from the Office of Naval Research, conducted a series of three workshops on the general theme, "Biotechnology: The Forging of Multidisciplinary Strategies for Research." The workshops were designed to cover areas of common interest to the Navy and North Carolina scientists and engineers. Each workshop explored the opportunities presented by the merging of biotechnology into a particular area; these three areas were (1) Biomolecular Electronics, (2) Materials Sciences, and (3) Microecology.

The series of workshops had two main objectives:

1. To discover, through experimenting with this strategy, the best approach for melding the perspectives of biologists, chemists, physicists, and engineers into a cohesive whole, and

2. To develop outlines for feasible interdisciplinary research programs.

The NCBC worked with three planning committees, one for each workshop, in planning and conducting the series. Approximately thirty scientists and engineers attended each two-day workshop. They came from the North Carolina Triangle institutions -- Duke University, University of North Carolina at Chapel Hill, North Carolina State University, and the Research Triangle Institute -- as well as from East Carolina University, North Carolina Agricultural and Technical State University, University of North Carolina at Charlotte, and Wake Forest University/Bowman Gray School of Medicine. Participants included, among others, several molecular biologists ("gene-manipulators"), microbiologists, protein and physical chemists, and engineers in fields ranging from biomedicine to fermentation to electronics.

Each workshop group produced a set of recommendations for future research. They are listed below by time frame and by general subject category. "Near term" research is regarded as being achievable in less than five years, "middle term" research is of sufficient complexity to require five to ten years, and "long term" research entails goals so challenging that more than ten years will be needed. Of course, research designated as "middle" or "long term" will only be productive if it is begun now. A more complete account of the planning for and the discussions in each workshop is provided in Chapters II - IV in the body of the report.

# BIOMOLECULAR ELECTRONICS

## 1. Near Term Research Recommendations

a. Organic and Metalloorganic Analogs to the Materials Currently Employed in Microelectronics:

i. Synthesize organic and metalloorganic materials with high specific conductivity and well controlled semiconducting, superconducting, and magnetic properties, respectively.

ii. Study the stability of these materials and search for structures that resist degradation.

iii. Explore crystal growth, thin film deposition and patterning methods for these materials.

iv. Explore hybrid devices employing these materials and conventional electronic materials as components of known or novel types of devices.

v. Develop loaded organic scintillator detectors and direct readout organic dosimeters.

b. Molecular Switching and Storage Systems:

i. Initiate parallel readout.

ii. Conduct detailed physical and chemical research into natural switching, such as membrane switching phenomena.

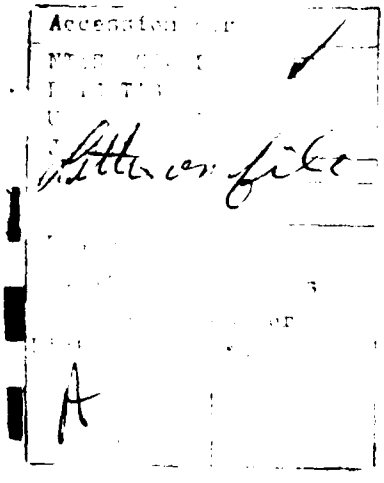
iii. Experiment with arrangement of molecular switching and storage elements in an addressable matrix.

iv. Determine average access time to be expected in a molecular storage device.

v. Determine limits to ultradense storage by organic devices due to power dissipation problems.

vi. Identify factors affecting the reliability of a molecular storage system and of its reproducibility in data processing.

vii. Define design goals to aid the organic chemists in developing active device elements; improve communication among semiconductor engineers, chemists, and biologists.



viii. Improve detection and readout techniques.

ix. Explore a broad spectrum of detectors, e.g., metalloorganics.

x. Synthesize and characterize a variety of molecules, such as synthetic porphyrins and metalloproteins, for efficient energy transfer.

xi. Examine the production of a molecular switching device.

xii. Develop an overall concept of system integration for a chemical computer, in order to guide the development of individual devices.

xiii. Study electron (or proton) transfer and tunnelling mechanisms.

c. Interfacing of Biochemical or Biological Systems and Semiconductor Devices:

i. Modify surfaces for corrosion protection, improve compatability (immune reactions), enhance the specificity and the optimization of charge transfer on semiconductor electrolyte junctions.

ii. Interface conductors or semiconductors with biochemical and biological model systems for fundamental studies on the control of biochemical processes by electrical or electromagnetic, including optical, stimulation.

iii. Study the fundamentals of tissue reaction occurring at device interfaces.

iv. Evaluate enzymatic and nonenzymatic, including synthetic, hemoproteins for electron conductivity in device fabrication and for laser sensitivity in device regulation.

v. Develop three-dimensional neuron cultures.

vi. Study directed epitaxial growth of bioorganic materials on appropriate substances.

vii. Electronically program cell growth (or culture) for specific purposes such as the production of specific molecules for export.

2. Middle Term Research Recommendations:

a. Organic and Metalloorganic Analogs to the Materials Currently Employed in Microelectronics:

i. Assess the potential of synthetic molecular substitutes for elementary and organic semiconductors, metals, and superconductors.

b. Molecular Switching and Storage Systems:

i. Initiate simple molecular computer experiments.

ii. Investigate miniaturization of chemical energy storage.

c. Interfacing of Biochemical or Biological Systems and Semiconductor Devices:

i. Explore in increasingly sophisticated manner the interactions between solid-state electronics and living systems.

ii. Demonstrate the feasibility of the transfer of a neuronal action potential through an electronic conductor to complete the information pathway.

iii. Develop a matrix of electrodes and explore intelligent high density circuits at the electrode site.

iv. Use single cells, such as algae or bacteria, or even fragments of cells, in sensory devices.

v. Match sensitive, selective phenomenon detection with electrical readout in electronic devices.

3. Long Term Research Recommendations. The long-term research recommendations are predicated upon advancements in the near and middle terms in the areas listed above. Consequently, these recommendations cross the boundaries of the three major areas discussed above. Therefore, they are simply listed below without assignment to any one category.

a. Analyze the integration of biomolecular systems with microelectronics devices for the design and production of a variety of new devices.

b. Analyze central nervous system mechanisms for processing information in terms of adaptation to sophisticated chip design.

c. Develop technologies for molecular switching and storing (following basic research in these areas).

d. Develop a molecular or hybrid semiconductor/molecular computer.

MATERIALS SCIENCES

1. Near Term Research Recommendations

a. Chemical Feedstocks, Energy, and Production of Chemicals:

i. Identify the problems associated with the scale-up of processes from laboratory demonstrations to commercial production. These problems include the special characteristics of large scale reactors and the influence of factors such as pH, surface conditions, and shear rate.

ii. Develop protocols for the use of genetically engineeered microorganisms in large scale fermenters.

iii. Study the stability of altered genetic information and its expression in large scale situations. Determine methods for maintaining the proper genetic strains (population dynamics).

iv. Investigate the use of immobilized antibodies for purification of enzymes and other separation processes.

v. Improve techniques of scale-up of tissue culture (both animal and plant) which, particularly when dovetailed with genetic engineering, can make possible mass production of desirable, rare chemicals, as well as increased rate and yield of biomass production.

vi. Produce a variety of natural products (desirable proteins) using genetic engineering. Examples include: glycoproteins, esterases for detoxification, barnacle glue, Limulus pyrogen detectors, calmodulin enzyme.

vii. Investigate gene insertion into blue-green algae (Cyanobacteria) which can provide their own energy requirements through photosynthesis.

b. Surface Interactions of Materials and Biological Systems:

i. Identify materials used by biological systems to adhere under varying conditions.

ii. Improve the understanding at the molecular level of microbial adherence.

c. Structure, Function, Design and Synthesis of Biological Macromolecules:

i. Clone and modify a gene to obtain a modified protein.

ii. Elucidate structure/function relationships through new genetic techniques.

2. Middle Term Research Recommendations

a. Chemical Feedstocks, Energy, and Production of Chemicals:

i. Explore the use of tissue culture techniques for obtaining new desired structures, such as films or fibers.

ii. Determine how to control the factors that have been identified as affecting scale-up of processes using genetically engineered organisms.

iii. Continue research and development on separation processes that will be essential to obtain pure products from the biological reactors.

iv. Identify organisms that possess both characteristics desirable in fermentation engineering (such as metabolic and genetic stability, solvent compatibility, tolerance of industrial stress, or longevity) and at the same time are suitable for genetic manipulation.

v. Improve the understanding of mutation dynamics in the scale-up of fermentation populations (population genetics).

b. Surface Interactions of Materials and Biological Systems:

i. Clone and transfer genes for barnacle glue, as a natural product of potential interest.

ii. Elucidate the substructure of proteinaceous films on surfaces; this has a wide variety of potential applications: for example in coatings, from repulsion of organism adherence to reduction of drag.

iii. Design adherent microorganisms that could occupy sites and block further adherence.

c. Structure, Function, Design and Synthesis of Biological Macromolecules:

i. Achieve significant understanding of enzyme active sites and functions, with special emphasis on active models of active sites of metalloenzymes.

ii. Design protein structures.

iii. Clone and modify a gene to obtain a modified protein, particularly proteins that are difficult to work with for a variety of reasons, e.g. exhibiting poor expressability.

3. Long Term Research Recommendations

a. Chemical Feedstocks, Energy, and Production of Chemicals:

i. Develop sustainable mixed populations of organisms possessing different, complementary capabilities.

ii. Increase the understanding of broad principles of processes of gene expression and control that may be generalized across species. This research should include studies under a variety of environmental conditions.

iii. Carry out the engineering research of scale-up such that a wide variety of novel products can be economically produced by a range of genetically engineered microorganisms to replace or augment currently expensive materials. Products based upon multiple genes for their synthesis may well necessitate use of biologically organized structures (e.g. orientation of a pathway on a membrane).

iv. Develop cell culture reactors.

v. Develop more cost-effective biological routes for energy sources (e.g., biomass, hydrogen, direct fuel cells) to replace partially petroleum feedstocks.

vi. Incorporate the requirements of scale-up in basic genetic studies of populations of single and mixed species.

b. Surface Interactions of Materials and Biological Systems:

i. Improve the understanding of the behavior of polymers at surfaces (interfaces), including absorption, adhesion, permeability, and ordered crystallization.

c. Structure, Function, Design and Synthesis of Biological Macromolecules:

i. Design protein and enzyme structures at the three-dimensional level.

ii. Design enzyme active sites.

iii. Develop artificial catalysts.

iv. Design macromolecules for detoxification of materials.

v. Incorporate into materials such features of biological systems as complex organization, flexibility, and adaptability; this will depend upon design based on understanding at the whole organism as well as at the molecular level.

## MICROECOLOGY

1. Near Term Research Recommendations

   a. Biological Consumption of Gases:

      i. Identify biocatalysts and marine microorganisms with catalytic properties of interest. Examine performance of ferric heme proteins and other enzymes in the catalysis of reactions of molecular hydrogen and other gases.

      ii. Determine the hydrogen metabolism of the blue-green algae and chemoautotrophic bacteria, and the mechanisms regulating its control.

   b. Chemoreception:

      i. Identify compounds and phenomena that act as signals to stimulate chemotaxis in marine microorganisms.

      ii. Identify receptor sites in marine microorganisms.

      iii. Determine the specificity and sensitivity limits of chemoreception.

   c. Organisms and Interfaces:

      i. Investigate biofilms.

      ii. Compare metabolic processes of free-living organisms and organisms at interfaces.

      iii. Study the colonization of surfaces by a variety of organisms; identify characteristics of various surfaces that stimulate or inhibit colonization.

   d. Products of Organisms:

      i. Catalogue and characterize natural products of marine organisms that might yield useful materials.

   e. Definition of Microecology:

      i. Identify useful products and capabilities arising in biological systems in the marine environment, such as biofilm coatings, drag reduction polymers, and detection "devices".

2. Middle Term Research Recommendations

a. Biological Consumption of Gases:

i. Investigate comparative enzymology and engineering properties of candidate organisms, including catalytic capabilities, kinetics and stability, and manipulability of rates and yields.

ii. Compare the microbial physiology, reaction mechanisms, and population dynamics of chemoautotrophs and photoautotrophs to determine their suitability as effective systems for elimination of unwanted substances.

iii. Define operational mechanisms for the biological consumption of hydrogen.

b. Chemoreception:

i. Investigate the molecular basis of chemoreception and of the function of receptor sites.

ii. Study the molecular basis of transduction of signals and response to physio-chemical signals to determine what connects receptor proteins to flagellar motility.

iii. Determine how organisms discriminate among a multitude of signals in a "noisy" environment.

c. Organisms and Interfaces:

i. Develop synthetic polymers based on responses of microorganisms to interfaces. Increase knowledge of basic mechanisms of attachment of organisms, including metabolism, physical and chemical bonding, influence of nutrients, and differences between organic and inorganic surfaces. This may have ramifications at various interfaces of the ocean environment in a variety of problems, not the least of which is fouling.

ii. Study the nature of reversible and irreversible binding to surfaces.

d. Products of Organisms:

i. Identify and manipulate genes that code for desired natural products of marine organisms.

e. Definition of Microecology:

i. Investigate the mechanisms of chemical and biological processes in the marine environment.

3. Long Term Research Recommendations

a. Biological Consumption of Gases:

i. Use living biological systems involving blue-green algae and chemoautotrophic bacteria to accomplish consumption of hydrogen or other gases.

ii. Elucidate molecular principles of biocatalysis sufficient to allow use of engineered biocatalytic systems for control of gaseous pollutants.

b. Chemoreception:

i. Investigate the genetic basis for chemoreception and chemotaxis, and the genetic engineering of these capabilities.

ii. Explore the interface of chemotaxis mechanisms with biomolecular electronics in detection devices.

iii. Use chemotaxis mechanisms as a tool with which to study processes and change in the ocean environment.

c. Organisms and Interfaces:

i. Use organisms on surfaces of electronic devices to function as sensors.

ii. Use functions and structures modeled upon interactions of microorganisms with surfaces in sensors.

iii. Engineer microorganisms genetically for desired properties of attachment to particular interfaces.

d. Products of Organisms:

i. Develop commercial, medical, scientific, and military applications of products initially derived from marine organisms and then genetically engineered. The objectives of the Materials Sciences Workshop may well be pertinent here.

e. Definition of Microecology:

i. Continue basic research on mechanisms and processes that constitute the complex ecology of the ocean.

ii. Increase the understanding of what regulates the diversity of organisms present in the ocean. This would include identification of numbers and types of organisms present, as well as knowledge of their metabolic processes and products -- generally, the response of microorganisms to the known variables in physicochemical ocean parameters such as light, pressure, or temperature.

iii. Elucidate how organisms function: with techniques now on hand, including the new molecular tools, it should prove possible to move from previous monitorings of distribution of organisms to such knowledge. Regulation of processes will be of key importance.

## COMMON RESEARCH THEMES

Some areas of research emerged as central to the future development of all three fields considered by the workshops. They are:

1. Properties of Surfaces and Interfaces. More needs to be known about the mechanisms by which orgranisms recognize, adhere to, penetrate and damage surfaces. Greater understanding of the properties of interfaces between biological systems and inorganic materials is necessary if "hybrid" systems are to be developed to function as sensors and as switching and storage devices.

2. Molecular Structures and Production of New Materials. A greater understanding of molecular structures, of structure-function relationships in macromolecules, of protein active sites, and of gene expression is necessary to meet the specific needs in each of the three areas.

3. Understanding Biological Systems. Whether designing new electronic devices, a robot, or a new glue, the workshop participants emphasized the valuable insights that can come from the biological systems that perform similar functions. It is necessary, therefore, that research at the organ, organism, population, and system levels be conducted at the same time that increased efforts are underway to understand molecular processes.

4. Multidisciplinary Collaboration. Perhaps the clearest and loudest common message emerging from the workshops was the statement that multidisciplinary research groups are required to advance the frontiers of knowledge in all areas.

Beyond the research areas identified in the summaries above, the participants in the workshops recommended that support be provided for the training of research scientists who will be able to cross conventional academic boundaries in pursuit of these research objectives. They also discussed the needs for the scientific equipment that is essential for progress in these areas. Finally, there were strong recommendations that the groups gathered for these conferences should continue to convene. Many of the scientists were introduced to new techniques and some collaborative efforts have already begun as a result of interactions at the workshops. The North Carolina Biotechnology

Center will continue to work toward fostering further collaboration among investigators who attended these Navy-sponsored workshops, with the expectation that significant results can be obtained from these collaborations. More complete information on training and equipment needs and on possible steps to facilitate multidisciplinary research is provided in Chapter V in the body of the report.

## PREFACE

The workshops reported on in this document were an experiment -- an experiment in convening scientists and engineers from diverse backgrounds to examine new areas of science and technology and formulate concrete recommendations for future research, and an experiment in the effectiveness of a new state-based agency, the North Carolina Biotechnology Center, in working with university scientists and engineers in planning and conducting such a multidisciplinary process. The results are most encouraging and point the way to considering how to refine and strengthen these organizational and institutional innovations so as to encourage continued collaboration in carrying out the research recommended. The Biotechnology Center and the participating scientists and engineers are committed to the pursuit of this goal.

The success of the workshops was due in large part to the nature of the interactions among all those involved. No single individual, group, or organization controlled the action. Rather the key functions were shared among all the members of the Planning Committee and their institutions. Maintaining such a mode of operation over the long term will be essential to any multidisciplinary, multi-institutional group that aspires to pursue the research areas recommended in this report.

The Planning Committees could not have fulfilled their responsibilities without the prompt and able cooperation from all of the participating scientists and engineers. They constituted the program; their creative questions and suggestions are the bases for this report. For elaboration of any point

presented in this report, the reader is urged to contact any member of the planning committees or any participant.

The entire process, from the first meeting of a planning committee to the final production of this report, would not have been possible without the warm, conscientious, and efficient support of Charmayne Ange and Carolyn Elliott of the North Carolina Biotechnology Center. Finally, we are most appreciative of the financial support provided by the Office of Naval Research and of its confidence in our ability to plan and conduct the workshop series in a relatively short time period.

Planning Committee

December, 1982

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# I. INTRODUCTION

Biotechnology or biomolecular engineering is an evolving field of immense and diversified potential. Those looking ahead to the future of technological innovation have perceived that integration of recent findings in molecular biology with a variety of other lines of research can lead ultimately to important breakthroughs. The challenge is to achieve this integration. How can scientists who differ in expertise and professional perspectives communicate with each other sufficiently to forge the multidisciplinary research projects necessary for the potential of biotechnology to be fulfilled?

The only way to meet that challenge is to move ahead and take the first step, to experiment and "learn by doing." Interested research workers from different disciplines must be brought together and be provided with a common focus for exploration, with a definite objective in view. Only open, focused dialogue among scientists knowledgeable about the constituent parts of a complex problem can lead to the truly multifaceted approach necessary to the solution of the entire problem.

## A. North Carolina and the North Carolina Biotechnology Center

North Carolina is taking several steps towards bringing this philosophy into reality. Over 150 scientists in fields related to biotechnology appear in the inventory compiled by the North Carolina Biotechnology Center (NCBC)[1].

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[1] North Carolina Scientists in Fields Related to Biotechnology, North Carolina Biotechnology Center (Research Triangle Park, N. C., 1982).

University researchers in the engineering schools and physical science departments in the state, in addition to the medical and agricultural schools, are becoming increasingly captivated by the challenges of biotechnology-related problems. Scientific expertise is abundant in North Carolina; equally important, the willingness to experiment with multidisciplinary approaches is present. The North Carolina Biotechnology Center itself has as one of its major functions the stimulation of interdisciplinary research related to biotechnology.

The NCBC was established by Governor James B. Hunt, Jr. in November, 1981, as a unique, statewide, comprehensive effort to stimulate the development and application of biotechnology within the state. The Center is a flexible arrangement deliberately designed to experiment and "learn by doing." It encourages and facilitates interactions among researchers, universities, industry, venture capital groups, and government. As a catalyst, the Center has the following objectives:

1. To enhance the awareness and understanding throughout the state of the nature of the exciting advances in biotechnology, their potential and limitations, and of the requirements for the state to realize the maximum benefits from these advances.
2. To facilitate and stimulate the interdisciplinary and interinstitutional collaborations that will put North Carolina among the leaders in terms of the quantity and quality of resources and expertise available to work together on challenging problems in biotechnology.
3. To increase the interest of industry -- both new and existing companies -- and financial institutions in the potential of North Carolina in biotechnology and to involve these parties in the programs of the Center and of our colleges and universities.
4. To create an environment in which individuals in our academic institutions, state government, industry, and other institutions are challenged to initiate activities that help the state strengthen its capabilities in biotechnology and to assist these individuals, as appropriate, in the implementation of their activities.

B. U. S. Navy Sponsorship of the Workshops

In accordance with these objectives, the North Carolina Biotechnology Center, working with university scientists, prepared a proposal to the Office of Naval Research, U. S. Navy, requesting partial support for a series of three workshops on the general theme, "Biotechnology: The Forging of Multidisciplinary Strategies for Research." The workshops were designed to cover areas of common interest to the Navy and North Carolina scientists and engineers. Each workshop explored the opportunities presented by the merging of biotechnology into a particular area; these three areas were (1) Biomolecular Electronics, (2) Materials Sciences, and (3) Microecology. This document is the report of these workshops, held September 15-16, September 30-October 1, and October 18-19, 1982.

C. Objectives

The series of workshops had two main objectives, one having to do with a process and the other with a product. The objectives were:

1. To discover, through experimenting with this strategy, the best approach for melding the perspectives of biologists, chemists, physicists, and engineers into a cohesive whole, and
2. To develop outlines for feasible interdisciplinary research programs in each of the three areas.

D. The Process: Planning and Conducting the Workshops

Initially, the North Carolina Biotechnology Center convened a small, multidisciplinary working group of scientists and engineers to work with it in outlining the general approach to the workshops and in preparing the proposal submitted to the Navy. This working group, supplemented by additional experts on the workshop topics, was divided into three planning committees, one to do

the detailed planning for each workshop. The Center staff were members of each planning committee; the Center served as the convenor and the secretariat for each committee. The planning groups developed the program for each workshop such that the critical topics were woven into a coherent framework, identified and invited the speakers and participants, and prepared a workshop packet that was sent to all participants in advance of the session. This packet included an agenda, abstracts of the prepared presentations, and curricula vitarum of all participants together with a statement of the purpose of the workshop, a list of questions to guide the formulation of research recommendations, and a copy of the proposal to the Navy on which the workshop was based.

Each workshop consisted of an intensive two-day retreat at a remote conference center in the Research Triangle area. Approximately thirty scientists and engineers attended each workshop, drawn from the North Carolina Triangle institutions -- Duke University, University of North Carolina at Chapel Hill, North Carolina State University, the Research Triangle Institute, and the Microelectronics Center of North Carolina -- as well as from East Carolina University, North Carolina Agricultural and Technical State University, University of North Carolina at Charlotte, and Wake Forest University/Bowman Gray School of Medicine. Participants included, among others, several molecular biologists ("gene-manipulators"), microbiologists, protein and physical chemists, and engineers in fields ranging from biomedicine to fermentation to electronics.

All the workshops consisted of a mix of working sessions and informal interactions during meals and social periods. The specific format of each workshop was different, however, reflecting the nature of the topic, the stage of development of the area of investigation, the character of opportunities for

merging with biotechnology, and the particular expertise of participating scientists and engineers.

## E. Organization of Report

The report includes a separate chapter on each workshop, Chapters II, III, and IV. Each chapter is organized as follows. The ideas and problem areas that formed the basis for the workshop are described, along with the agenda. Research opportunities are then presented according to the subject category under which they were discussed in the workshop. Based on these research opportunities, specific research recommendations are presented according to time frame (near term, middle term, long term). Finally, there is a brief concluding section that highlights a few general points that are relevant to the implementation of the research recommendations.

Chapter V is an attempt to look across all three workshops and extract conclusions applicable to all. A few research categories appear to be central to all three areas. For the most part, however, issues of training, equipment needs, and the encouragement of multidisciplinary research receive most attention in this final chapter.

Workshop agenda, abstracts of prepared presentations, and biographical information on the participants are included in the Appendices.

# II. BIOMOLECULAR ELECTRONICS

## A. Focus on Four Problem Areas

The purpose of this workshop was to explore development of multidisciplinary approaches for successful research in the merging of biotechnology and biomolecular electronics. In order to focus the thinking of the participants, the general topic was broken down into four problem areas. The substance and rationale for each is described below.

1. Organic and Metalloorganic Analogs to Semiconductor Devices. The need for future ultrahigh speed computers in artificial adaptive systems requiring very high density storage has generated considerable interest in biomolecular analogs to the current semiconductor device technology. Thus, problems can best be tackled by reviewing the existing knowledge in this area and bringing together biologists, chemists, engineers and physicists who have either ongoing programs or an interest in joining fundamental research efforts aimed at organic conductors and semiconductors, molecular switching and storage, and microfabrication techniques that allow integration and addressing of nanometer scale devices.

2. Interaction of Radiation with Organic Materials and Biochemically Active Surfaces. Organic scintillators and new areas of organic semiconductor detectors should be considered. The alterations in optical and other physical or electronic properties of organic materials resulting from radiation damage may allow new uses, such as in direct readout dosimeters or other devices. Picosecond laser spectroscopy, optical activation, and photon, ion and electron beam processing of surfaces are important areas for consideration.

3. Integration of Biomolecular Systems with Microelectronic Devices. The full utilization of the potential of biomolecular switching and storage devices requires three-dimensional integration employing radically different interconnection principles and I/O schemes. In the next decade we may expect an increasing degree of hybridization between biological and microelectronic systems with substantial payoff in the investigation of fundamental phenomena in biotechnological subsystems and exciting opportunities in photocatalysis and biomedicine. Consideration needs to be given to research aimed at electric contacts to active groups of proteins, semiconductor analogs of photosynthesis, and passivation and corrosion phenomena of semiconductors in a biochemical environment.

4. Biomolecular Circuits and Systems. As very large scale integration (VLSI) and continually smaller device structures are developed, associated problems arise and must be addressed. The conventional clean separation of device design from system design depends on being able to isolate individual devices from the environment of the other devices, except for planned effects occurring through desired interconnections. In very dense arrays of devices, line to line capacitance and nearest neighbor interactions begin to dominate the system. Thus, unwanted device or system characteristics may arise. Three-dimensional structures as utilized in living systems have the potential for illustrating solutions to the high density device interaction problem. System characteristics and parallels with neural tissue (extremely high connectivity, redundancy and adaptive properties) may well prove very helpful in understanding these problems. The potential application of macromolecular and multicellular systems to biomolecular electronics is great in terms of size reduction, power reduction and the fabrication of devices with enhanced performance characteristics.

The agenda was organized into four three-hour sessions, one devoted to each of the problem areas. Each session consisted of four prepared presentations and a one-hour period to consider conclusions and recommendations for future research. In addition, there were introductory remarks by the Governor's Science and Public Policy Advisor, and a representative of the Navy, an evening talk by the President of the Microelectronics Center of North Carolina, and an overview of advances in molecular microelectronics by Dr. Forrest Carter of the Naval Research Laboratory. A discussion of interdisciplinary collaboration in training and of research needs took place during the evening as well. The complete agenda together with abstracts of the prepared presentations are included in Appendix A. Curricula vitarum of all workshop participants are included in Appendix D.

The initial paper presentations in each session were intended to elucidate some of the technical issues and opportunities associated with the problem area. During the concluding sessions, the participants -- using

the ideas presented and drawing upon their diverse experiences -- formulated recommendations for research projects and programs that deserve high priority. The questions below were used as guidelines in reaching these conclusions, which are described in the next section.

- In what research areas are biology and microelectronics already merging, and what do these interfaces suggest concerning the symbiotic roles of modern biological and chemical understanding and solid state electronics in the future of biotechnology?
  - What are the current and foreseeable problems, limitations and opportunities presenting the most serious challenges to biomolecular electronics?
  - What solutions do biological systems and synthetic analogs provide to these problems?
- What research pursuits hold the most promise in the near-term (less than 5 years), middle-term (5 to 10 years), and long-term (beyond 10 years) future?
- How is this research, along with concomitant training, to be supported and conducted in order to (1) ensure the necessary communication and collaboration between researchers with a biological perspective and researchers with physical, chemical or engineering perspectives, and (2) ensure the continuity of investigation necessary to achieve these complex goals?

B. Research Opportunities

Research opportunities perceived by the participants as deserving special attention are outlined briefly below, according to the workshop session in which they arose.

1. Organic and Metalloorganic Analogues to Semiconductor Devices

   a. Substitute materials for the fabrication of conventional electronic devices were considered. Organic and bioinorganic materials with metallic and semiconductor behavior have been synthesized, and superconductivity for organic materials has been demonstrated. However, these materials currently have lower transport rates, critical temperature and are chemically less

stable when compared with the inorganic conductors, semiconductors and superconductors used in the fabrication of today's solid state electronic devices. Key problems that need to be investigated are:

i. Stability to degradation, and

ii. A more meaningful characterization of new materials, which requires more work on single crystal specimens. Properties that could give synthetic molecular structures a competitive edge are the option of built-in magnetic structure and of anisotropic carrier mobility. Although much can be learned in this area in the near future, a reasonably complete assessment of the potential of synthetic molecular substitutes for elemental and compound semiconductors, metals and superconductors cannot be expected in less than 10 years.

b. Novel device structures based on new materials, i.e., molecular switching and storage devices, also deserve attention. Although molecular switching and storage devices have the potential of ultrahigh storage density ($10^{15}$-$10^{18}$ bits/$cm^3$), many questions must be answered before their technological potential can be assessed:

i. How can molecular switching and storage elements be arranged in an addressable matrix?

ii. What is the average access time that we may expect in a molecular storage device?

iii. What are the limits to ultradense storage by organic devices due to power dissipation problems?

iv. What are factors that affect the reliability of a molecular storage system and its reproducibility of data processing?

v. Can novel synthesis methods be developed to provide for the assembly of angstrom size structures on substrates? The advantage of self assembly of biomolecular systems is considered to be essential for fabricating the complex three-dimensional structure needed for molecular computing systems.

Preliminary answers to at least some of these questions may be possible on a near term basis, but in terms of technological expectations, research in molecular switching and storage must be viewed as a long term investment.

c. The interrelation between biological information-processing systems and semiconductor devices calls for further study. The utilization of equivalent circuits incorporating passive and active semiconductor elements can be helpful in modeling biological information-processing elements. However, the switching mechanisms in such systems are not sufficiently understood, and detailed studies of the chemistry and physics of membrane switching phenomena are needed. The highly specific nature of antibodies or enzymatic reactions and the established capabilities of semiconductor circuits in signal processing, transmission and storage could be combined in the development of highly sensitive hybrid biomolecular-solid state electronic sensors for biological or biochemical events. Detailed data concerning the behavior of natural switching systems will be important for the design of such hybrid devices. Although biological transmission and storage is slow compared to existing solid state devices, the overall biological system performance is fast; and we may learn from the analysis of biological systems how to optimize the design of highly integrated semiconductor systems. An understanding of the design of optical biological systems, for example, may influence our thinking in solid state integrated optics design.

2. Interaction of Radiation with Organic Materials and Biochemically Active Surfaces

a. One central question concerned the characteristics of molecular structures that govern their electron transport properties. The laser activation of electron transport in metalloporphyrins was discussed. Instead of forcing an analogy with solid state devices, a more productive approach would be to attempt to identify clearly and then to utilize special features of each system in appropriate combinations. This will require careful yet imaginative definition of those desirable functions that the resultant devices will be asked to provide. Thus, a key activity in the next few years will be enhanced communication between semiconductor engineers and biologists, so that awareness is shared as to the ways in which different systems meet a variety of needs.

b. Other more specific areas that surfaced in discussions include the following:

i. Improvements in detection and read-out techniques are needed, both biological and non-biological fingerprinting. A broader spectrum of detectors, e.g., metalloorganics and metalloproteins, should be opened to exploration.

ii. Studies of a wide range of electron conducting macromolecules should be carried out and the role of solvent

systems should be examined. Synthesis and characterization of a variety of molecules suspected of efficient energy transfer hold great promise.

iii. Another area of fundamental importance involves communication between semiconductors and the outside world. Two avenues that should receive attention are optical coupling and biochemical coupling.

c. Surface analytical techniques hold great promise for making major contributions to advances in biomolecular electronics. Many in the group were not aware of these techniques prior to the workshop, yet from the discussion of them arose many areas of common research interests. Analytical chemists and others with such expertise, therefore, need to be involved in further pursuits.

3. Integration of Biomolecular Systems with Microelectronic Devices

a. In the introductory talk by Dr. Carter in the first session, the idea of a molecular computer and criteria for its design were presented. It was demonstrated how three-dimensional organic computing elements on the surface might be accomplished. Examples of the potential molecular switching devices based on the concept of the soliton were given. It was demonstrated how in conjugated organic molecules soliton waves could be used for information switching and storage, and how the molecules might be assembled to provide molecular logic circuits. In the discussion it was mentioned that molecules are intrinsically random elements. Nature makes reliable switching devices from randomly switching molecules, and we might learn from biological systems how to accomplish this. It was stressed that it will be necessary to learn the techniques of self-synthesis and self-organization from both synthetic chemistry and the biological world. The advantage of the molecular computer lies largely in size reduction, where $10^{15}$ active units per $cm^3$ could be possible. The need exists to develop design goals to aid the organic chemist in developing active device elements. An overall concept of system integration for a chemical computer should be developed early on to guide the development of actual devices to insure a reasonably integrated and therefore functional system.

b. The idea of charge transfer by electron tunnelling from a semiconductor device through a thin film to an electrolyte solution was developed. The critical element relates to the thin film material which determines both the corrosion resistance and charge transfer properties. Such a device may be used to trigger chemical processes or possibly for electrical stimulation. Basing the device on a semiconductor substrate, therefore makes possible the development of a matrix of electrodes and the possibility of intelligent high density circuits being at the electrode site. Fundamental research to identify transfer

mechanisms would be required in pursuit of this objective. Problems associated with such an electrode, particularly in terms of biological tissue reactions, could possibly be minimized by using the technique presented for modification of inorganic surfaces, in this case glass beads, for immobilization of biological molecules. (Treating the beads provides a technique which can be used to enable embedding of antibodies or fragments on the surface in order to prevent tissue reaction.)

c. The covalent attachment of bio-organic molecules to non-metallic microelectrodes was suggested, specifically using single carbon fibers, seven microns in diameter. The ability to bind catecholamines or larger molecules such as cytochrome-C were illustrated. It was suggested that electron transport in some way, possibly by tunnelling, can pass electrons through large molecules. Thus, this technique for shielding a thin-film-coated, solid-state, stimulating device may not significantly change its properties. A technique was discussed for electron beam lithography on methacrylate-coated polylysine followed by the application of cultured neurons and the laying down of conducting materials. This would permit an electrically active interface between the cultured neurons which stick to the substrate and the conducting material. A critical step will be to demonstrate that the information which is carried in the form of an action potential can be transferred to an elecronic conductor to complete the information pathway.

d. In general, the integration of biomolecular systems with microelectronic and optoelectronic devices has the potential for developing sensors of high specificity and sensitivity for both detecting biological events and monitoring biotechnological processes. Also, it offers the possibility of regulating biological and biochemical systems. Research in these areas can be successful in the near term, and will contribute in the long term to the development of molecular computers.

4. Biomolecular Circuits and Systems

a. Possible biomolecular circuits were considered. Nonenzymatic hemoproteins could be important organic compounds to be evaluated for electron conductivity in device fabrication. They should also be evaluated for laser sensitivity in device regulation.

b. The degree of interaction among individual circuit components must be considered, as well. The mammalian central nervous system, because of its high connectivity and complex electrical and chemical organization, can be a very important model for sophisticated chip design. New strategies and architecture of computer design may be developed based on the organization of the brain. The techniques employed by the central nervous system to control unwanted activity, such as occurs in the epilepsies, may

be a guide to controlling undesired characteristics that will undoubtedly appear in high density, three-dimensional computer circuits. Along this line, neuron cultures should be developed such that they can interact with, or contact, each other three-dimensionally. It appears that epitaxis may be very important in directing the growth and structure of the biological devices. More research is needed on guided epitaxial growth of bio-organic materials on appropriate substrates.

c. A full arsenal of techniques originating from a variety of disciplines must be marshalled for future shared endeavors. Less expensive ways of accomplishing electron beam lithography for the design and manufacture of smaller devices should be sought. Alternative, less complicated methods, such as phase contrast microscopy should be evaluated for device inspection when tissue-chip interfaces are involved.

d. Overall, the potential for complex molecular and multicellular systems to provide insight into high density, three-dimensional circuit strategies is great, and extensive efforts should be directed toward understanding cultured cell assemblies in the near term and central nervous system parallels over the middle term.

## C. Research Recommendations

From the discussions summarized in the previous section, specific research recommendations were identified. They are grouped here according to time frame and to three conceptual categories that emerged as being of central importance. "Near term" research is regarded as being achievable in less than five years, "middle term" research is of sufficient complexity to require five to ten years, and "long term" research entails goals so challenging that more than ten years will be needed. Of course, research designated as "middle" or "long term" will only be productive if it is begun now.

### 1. Near Term Research Recommendations

a. Organic and Metalloorganic Analogs to the Materials Currently Employed in Microelectronics:

i. Synthesize organic and metalloorganic materials with high specific conductivity and well controlled semiconducting, superconducting, and magnetic properties, respectively.

ii. Study the stability of these materials and search for structures that resist degradation.

iii. Explore crystal growth, thin film deposition and patterning methods for these materials.

iv. Explore hybrid devices employing these materials and conventional electronic materials as components of known or novel types of devices.

v. Develop loaded organic scintillator detectors and direct readout organic dosimeters.

b. Molecular Switching and Storage Systems:

i. Initiate parallel readout

ii. Conduct detailed physical and chemical research into natural switching, such as membrane switching phenomena.

iii. Experiment with arrangement of molecular switching and storage elements in an addressable matrix.

iv. Determine average access time to be expected in a molecular storage device.

v. Determine limits to ultradense storage by organic devices due to power dissipation problems.

vi. Identify factors affecting the reliability of a molecular storage system and of its reproducibility in data processing.

vii. Define design goals to aid the organic chemists in developing active device elements; improve communication among semiconductor engineers, chemists, and biologists.

viii. Improve detection and readout techniques.

ix. Explore a broad spectrum of detectors, e.g., metalloorganics.

x. Synthesize and characterize a variety of molecules, such as synthetic porphyrins and metalloproteins, for efficient energy transfer.

xi. Examine the production of a molecular switching device.

xii. Develop an overall concept of system integration for a chemical computer, in order to guide the development of individual devices.

xiii. Study electron (or proton) transfer and tunnelling mechanisms.

c. Interfacing of Biochemical or Biological Systems and Semiconductor Devices:

i. Modify surfaces for corrosion protection, improve compatability (immune reactions), enhance the specificity and the optimization of charge transfer on semiconductor electrolyte junctions.

ii. Interface conductors or semiconductors with biochemical and biological model systems for fundamental studies on the control of biochemical processes by electrical or electromagnetic, including optical, stimulation.

iii. Study the fundamentals of tissue reaction occurring at device interfaces.

iv Evaluate enzymatic and nonenzymatic, including synthetic, hemoproteins for electron conductivity in device fabrication and for laser sensitivity in device regulation.

v. Develop three-dimensional neuron cultures.

vi. Study directed epitaxial growth of bioorganic materials on appropriate substances.

vii. Electronically program cell growth (or culture) for specific purposes such as the production of specific molecules for export.

2. Middle Term Research Recommendations:

a. Organic and Metalloorganic Analogs to the Materials Currently Employed in Microelectronics:

i. Assess the potential of synthetic molecular substitutes for elementary and organic semiconductors, metals, and superconductors.

b. Molecular Switching and Storage Systems:

i. Initiate simple molecular computer experiments.

ii. Investigate miniaturization of chemical energy storage.

c. Interfacing of Biochemical or Biological Systems and Semiconductor Devices:

i. Explore in increasingly sophisticated manner the interactions between solid-state electronics and living systems.

ii. Demonstrate the feasibility of the transfer of a neuronal action potential through an electronic conductor to complete the information pathway.

iii. Develop a matrix of electrodes and explore intelligent high density circuits at the electrode site.

iv. Use single cells, such as algae or bacteria, or even fragments of cells, in sensory devices.

v. Match sensitive, selective phenomenon detection with electrical readout in electronic devices.

3. Long Term Research Recommendations. The long-term research recommendations are predicated upon advances in the near and middle terms in the areas listed above. Consequently, these recommendations cross the boundaries of the three major areas discussed above. Therefore they are simply listed below without assignment to any one category.

a. Analyze the integration of biomolecular systems with microelectronics devices for the design and production of a variety of new devices.

b. Analyze central nervous system mechanisms for processing information in terms of adaptation to sophisticated chip design.

c. Develop technologies for molecular switching and storing (following basic research in these areas).

d. Develop a molecular or hybrid semiconductor/molecular computer.

D. General Conclusions

The following observations are relevant to the implementation of the research agenda suggested in the previous two sections.

1. Approaches to new compounds and materials with special magnetic, optical, catalytic and electronic properties have been suggested in the various sessions. These synthetic materials mimic biological molecules and, in some instances, can have improved characteristics. Expertise in synthetic chemistry is required to pursue developments along these lines. The workshop revealed that such expertise exists in North Carolina.

2. Much can be learned from complex living systems in:

   i. The design of VLSI devices,

   ii. Directed organization and self assembly, and

   iii. Control of chemical reactions through cellular compartmentalization and membrane phenomena.

3. An important mechanism for the synthesis of complex organic structures involves the use of epitaxial deposition on substrates that match both chemically and geometrically, as, for example, in enzymatic reactions.

4. The successful communication between biological systems and semiconductors depends on a fuller understanding of the biological, chemical and physical properties of the interfacial region. This requires extensive collaboration among scientists of different disciplines. Such interaction was evident at the workshop and future collaborative research was stimulated.

# III. MATERIALS SCIENCES

## A. Focus on Four Problem Areas

The purpose of this workshop was to explore development of multidisciplinary approaches for successful research in the merging of biotechnology and the materials sciences. As in the biomolecular electronics workshop, the general topic was broken down into four problem areas in order to focus the thinking of the participants. The substance and rationale for each is described below.

1. Chemical Feedstocks, Energy, and Production of Chemicals. The chemical industry, once relying on a significant, renewable agricultural base for feedstocks, has been largely petroleum-based in recent decades. An examination of the reasons for this shift along with current factors which might lead to a stronger biological component, not only in terms of feedstocks but also of processing, may lead to a better appreciation of the potential in this realm. The potential contributions of specific areas of biology to the chemical industry, as well as the requirements for biology to make a significant impact, form a body of knowledge to guide further research.

2. Structure and Function of Biological Macromolecules. The examination of the structure of active sites of enzymes and possible approaches to their modification is a field of much promise for biotechnology. Also considered in this area is the role of ions, particularly metal ions, in protein function and structure. The whole area of the mechanisms of enzyme catalysis is ready for elucidation.

3. Surface Interactions of Materials and Biological Systems. The interactions between microorganisms and surfaces deserve careful study. Microorganims can stimulate higher organism adherence, as well as adherence by microorganisms themselves. Materials synthesized by microorganisms for such adherence may have applications. These observations lead to consideration of the destructive processes of microorganisms. The use of immobilized proteins for extraction of gases and the use of natural "glues" provide examples of biological problems in materials. The design apparent in naturally occurring marine structures may provide suggestions for design features to be deliberately synthesized.

4. Design and Synthesis of Proteins. The deliberate design and synthesis of proteins for particular purposes was a primary theme for much of this conference and is central to much of the thinking about the contributions of biotechnology to materials sciences. The capacity to engineer proteins, other biomolecules, or possibly organisms to possess particular properties, to make certain products, or to perform

particular functions may provide potential for future pursuits in the materials sciences.

Following the approach used in the Biomolecular Electronics Workshop, the agenda was organized into four three-hour sessions, one devoted to each of the problem areas. Each session consisted of several prepared presentations and a one-hour period to consider conclusions and recommendations for future research. In addition, the Governor's Science and Public Policy Advisor made some introductory remarks, and two representatives of the U. S. Navy discussed the Navy's interest in materials sciences after dinner. A discussion of future possibilities for interdisciplinary collaboration in training and research took place during the evening. The complete agenda together with abstracts of the prepared presentations are included in Appendix B. Curricula vitarum of all workshop participants are included in Appendix D.

The initial paper presentations in each session were intended to elucidate some of the technical issues and opportunities associated with the problem area. During the concluding sessions, the participants -- using the ideas presented and drawing upon their diverse experiences -- formulated recommendations for research projects and programs that deserve high priority. The questions below were used as guidelines in reaching these conclusions, which follow:

- In what research areas are biotechnology and materials sciences already merging, and what do these interfaces suggest concerning the symbiotic roles of molecular biology, biology, chemistry and physics in the future of biotechnology?
  - What are the current and foreseeable problems, limitations and opportunities presenting the most serious challenges to the materials sciences?
  - What solutions do biological systems provide to these problems?
- What research pursuits hold the most promise in the near term (less than 5 years), middle term (5 to 10 years), and long term (beyond 10 years) future?

- How is this research, along with concomitant training, to be supported and conducted in order to (1) ensure the necessary communication and collaboration between researchers with a biological perspective and researchers with physical, chemical or engineering perspectives, and (2) ensure the continuity of investigation necessary to achieve these complex goals?

## B. Research Opportunities

Research opportunities perceived by the participants as deserving special attention are outlined briefly below. They are organized according to the workshop session in which they were discussed.

1. Chemical Feedstocks, Energy, and Production of Chemicals. Important needs can be addressed in the materials production area through a blend of fundamental research and attention to special engineering problems of scale-up. Exchange of information and ideas between the biological and engineering sciences can lead to improved biological techniques for the production of useful materials of a much wider variety than is presently available. Specific areas of the necessary interdisciplinary research are identified below.

   a. Genetically improved microorganisms or tissue cells can provide significant advantages in terms of generation time, specificity of reactions, complexity of possible products, and sensitivity to environmental conditions. Changes in important parameters such as performance, yield, efficiencies, and specificity can be very dramatic with altered conditions. Therefore, if a whole range of genetically improved species is to be utilized effectively in energy, chemicals, and feedstock production, engineering research is needed on minimizing and controlling variables which have the largest impact on the performance of genetically improved species in scaled-up processes. The implications of changing conditions are so significant that conventional bioengineering scale-up procedures will probably be inadequate. Equipment and protocol development are needed to allow testing of species under different conditions. While some of this does exist, much more attention should be given to the conditions that are actually relevant to variations found in larger-scale processes. As one example of the need for communication between engineers and biologists, insight gained by engineers as to identification of the more important environmental variables should be communicated to those working in basic sciences, just as scientists need to convey information about biological production of potentially useful substances to engineers.

   b. An approach to ameliorating uncontrolled variations likely to occur is the development of mixed populations of a species, such that different populations contribute to the same overall

function but operate at different optima of certain variables (e.g., temperature). These mixed gene pool systems should be investigated with respect to their preparation and maintenance, possible interferences, and reactor design limitations. Research in population biology, particularly microbial ecology and population genetics, will contribute valuable insights here.

c. An improved species developed under a specific set of conditions does not necessarily express that altered genetic information when placed under other conditions, which may seem to be identical in terms of large scale indicators. Thus gene expression is of high priority as a subject not only for basic research, but for engineering research as well. Molecular understanding of gene expression and adaptation mechanisms is therefore a key requirement in the overall improvement of materials and chemicals production. This research could build on the relatively precise knowledge of microbial genetic processes. The traditionally more empirical techniques associated with tissue cell production also stand in need of extensive analytical investigation in terms of the molecular rules governing gene expression and cell differentiation. At present, knowledge of these more complex and multifunction cellular systems is limited, and basic tissue cell culture research is needed to increase the short and long term improvements for materials production.

d. Each of the identified research areas could benefit in the near term from increased research. The engineering areas may yield more significant short term results because of the current relatively low level of attention directed to scale-up problems. Thus the proportional impact of increased emphasis may prove to be very great. However, since the production of new chemicals by microbial or tissue cell processes requires a continuing relationship between basic research achievements and implementation on a large scale, with feedback regarding practical limitations, both types of research will be necessary for long term success of materials production via genetically improved biological techniques.

2. Surface Interactions of Materials and Biological Systems. Interactions of surfaces and organisms emerged as an area with many possible, significant ramifications.

a. Basic studies in materials sciences involving epitaxial deposition and absorption can help to illuminate the problems at biological interfaces. The application of specialized instrumentation to the study of surfaces can lead to valuable information about the nature of the interactions. Attenuated total reflectance infrared spectroscopy, inelastic electron tunneling spectroscopy, photoacoustic spectroscopy, Raman spectroscopy, and radiolabeled antibody absorption are tools that could be used by workers in this area.

b. Sub-molecular properties are of particular interest. For example, when an organism adheres to a structure, there is often a conditioning layer of ions, proteins and/or polysaccharides that mediates between the surface and the organism. The molecular nature of this conditioning layer needs to be understood and, perhaps more importantly, the nature of the 1-3 angstrom molecular structure at the interface needs to be characterized.

c. The diversity of surface interactions with organisms is illustrated by the following examples:

The range of complexity of the interactions of organisms with surfaces is demonstrated by the attachment of Agrobacterium tumefaciens, which uses a protein receptor to guide its attachment to a plant and then extrudes fibrils of cellulose to anchor itself and, through entanglement, guide other bacteria to the surface. Recently developed techniques for inducing mutations have allowed the development of bacterial strains which do not produce the anchoring fibrils of cellulose. Although they can carry out their functions under laboratory conditions, these bacteria are readily washed off in an environment that simulates "real world" conditions, thus demonstrating the critical role in adherence played by the genetically coded-for fibrils.

More complex organisms, such as the shipworm, recognize suitable sites for colonization on wooden structures in the sea by sensing that the wood has previously been invaded by marine fungi which partially break down the surface. Interruption of the highly destructive phase of wood consumption by the shipworm might be accomplished by interfering with the early, less destructive phases of fungal attachment. Thus, understanding of interactions among different organisms, involving molecular, whole organism, and ecological knowledge, may be necessary in order to appreciate the entirety of biological influence upon surfaces.

The pH dependent and nonlinear solubility of oxygen in various marine hemoglobins permits organisms to function in their specialized environments by facilitating oxygen transfer across gill surfaces. This property can be used to create practical mass transfer devices for extracting useful amounts of oxygen from seawater. Basic research on marine hemoglobins has made possible the selection of an optimum hemoglobin for this function and further suggests modifications of the hemoglobin structure which can lead to enhanced performance of the oxygen extractor. These modified structures may be obtained by chemical manipulation of hemoglobin or by genetic manipulation of the hemoglobin forming organisms.

d. A strong case was made for the importance of understanding the versatility and complexity of biological systems. The trend of the times is to study biology at the molecular and cellular level. This was seen by the group as appropriate but not sufficient. The new techniques, theories, and knowledge will inevitably lead to an avalanche of molecular and microbiological studies. There was consensus that along with these lines of inquiry continuous support should be provided for study of whole organism systems and animal diversity. For example, the many solutions to problems arrived at through evolution by natural selection offer a far richer range of models as approaches to robotics than would a simple, anthropomorphic mimicking of human functions.

3. Structure and Function of Biological Macromolecules

a. Considerable fundamental information is being accumulated about the active sites of proteins. Binding reactions are in general understood better than catalytic reactions. More fundamental research is needed to understand how active sites work, how proteins fold, and how proteins interact with solvents. The design of molecules with desired catalytic or binding properties requires a greatly increased understanding of basic mechanisms. Small molecule model catalysts can be made for some reactions, particularly those involving metal ions. The nature of active sites in proteins can be investigated both by modifying the sites chemically and by modifying the genes which code for the proteins.

b. Protein stability as well as activity can be modified, particularly by immobilizing the protein. Immobilized binding sites may also be useful for sequestering substances. If enzymes are to be used for practical purposes, their stability is a characteristic that will need to be thoroughly understood.

c. Proteins and biological systems, in comparison to metalloorganic catalysts, possess a high degree of specificity and selectivity. This specificity could be useful both in enzymatic and binding reactions. One area of application is the use of enzymes in microelectronic coding and switching. Detoxification is another possible area of application -- particularly in cases where specificity and high sensitivity are required. For example, this might be the case with trace metals and some trace organic compounds.

d. Biological systems can mediate crystal formation and perhaps could be used to obtain specific desirable conformations.

4. Design and Synthesis of Proteins

a. The mechanism of protein synthesis in bacteria is well understood. The requirements for this translation of genes inserted into prokaryotes have to be taken into account in cloning eukaryotic genes into bacteria. Mechanisms of protein synthesis in the eukaryote cytoplasm and in chloroplasts and mitochondria are less well understood. In order to insert new genes into eukaryotes and to have these genes successfully expressed, more knowledge of these mechanisms is needed.

b. The technology required to synthesize oligonucleotides of desired sequences and to clone these sequences is now widespread. This technology can be used to synthesize proteins of any desired amino acid sequence and thus to examine the amino acid sequence requirements for protein folding in forming active sites. Examples of proteins not readily studied by other technologies are the restriction endonuclease Eco RI and calmodulin. Both of these proteins can be studied using the synthetic oligonucleotide and cloning approach. A future direction would be the synthesis of a new protein of a desired sequence to fulfill a particular function.

c. The study of known protein crystallographic structures can be used to try to choose amino acid sequences which would have a desired three dimensional structure. More basic information on how proteins fold is needed. Many schemes for predicting secondary structure from sequences exist but these are only tentative predictions. The synthesis of proteins with a desired amino acid sequence is one method that could be used to study protein folding. The design of new proteins should allow the testing of factors involved in protein folding and activity. Binding sites would be easier to design than catalytic sites. As well as designing structure for study, special features such as reporter groups, and modifiable side-chains can be incorporated. The desire to design a molecule for a particular use as well as for study may expand the viewpoints of the investigators.

C. Research Recommendations

From the discussions summarized in the previous section, specific research recommendations were identified. They are grouped here according to time frame and to three major categories. The definitions for near term, middle term, and long term are the same as those used previously in Chapter II.C.

1. Near Term Research Recommendations

a. Chemical Feedstocks, Energy, and Production of Chemicals:

i. Identify the problems associated with the scale-up of processes from laboratory demonstrations to commercial production. These problems include the special characteristics of large scale reactors and the influence of factors such as pH, surface conditions, and shear rate.

ii. Develop protocols for the use of genetically engineered microorganisms in large scale fermenters.

iii. Study the stability of altered genetic information and its expression in large scale situations. Determine methods for maintaining the proper genetic strains (population dynamics).

iv. Investigate the use of immobilized antibodies for purification of enzymes and other separation processes.

v. Improve techniques of scale-up of tissue culture (both animal and plant) which, particularly when dovetailed with genetic engineering, can make possible mass production of desirable, rare chemicals, as well as increased rate and yield of biomass production.

vi. Produce a variety of natural products (desirable proteins) using genetic engineering. Examples include: glycoproteins, esterases for detoxification, barnacle glue, Limulus pyrogen detectors, calmodulin enzyme.

vii. Investigate gene insertion into blue-green algae (Cyanobacteria) which can provide their own energy requirements through photosynthesis.

b. Surface Interactions of Materials and Biological Systems:

i. Identify materials used by biological systems to adhere under varying conditions.

ii. Improve the understanding at the molecular level of microbial adherence.

c. Structure, Function, Design and Synthesis of Biological Macromolecules:

i. Clone and modify a gene to obtain a modified protein.

ii. Elucidate structure/function relationships through new genetic techniques.

2. Middle Term Research Recommendations

a. Chemical Feedstocks, Energy, and Production of Chemicals:

i. Explore the use of tissue culture techniques for obtaining new desired structures, such as films or fibers.

ii. Determine how to control the factors that have been identified as affecting scale-up of processes using genetically engineered organisms.

iii. Continue research and development on separation processes that will be essential to obtain pure products from the biological reactors.

iv. Identify organisms that possess both characteristics desirable in fermentation engineering (such as metabolic and genetic stability, solvent compatibility, tolerance of industrial stress, or longevity) and at the same time are suitable for genetic manipulation.

v. Improve the understanding of mutation dynamics in the scale-up of fermentation populations (population genetics).

b. Surface Interactions of Materials and Biological Systems:

i. Clone and transfer genes for barnacle glue, as a natural product of potential interest.

ii. Elucidate the substructure of proteinaceous films on surfaces; this has a wide variety of potential applications: for example in coatings, from repulsion of organism adherence to reduction of drag.

iii. Design adherent microorganisms that could occupy sites and block further adherence.

c. Structure, Function, Design and Synthesis of Biological Macromolecules:

i. Achieve significant understanding of enzyme active sites and functions, with special emphasis on active models of active sites of metalloenzymes.

ii. Design protein structures.

iii. Clone and modify a gene to obtain a modified protein, particularly proteins that are difficult to work with for a variety of reasons, e.g. exhibiting poor expressability.

3. Long Term Research Recommendations

a. Chemical Feedstocks, Energy, and Production of Chemicals:

i. Develop sustainable mixed populations of organisms possessing different, complementary capabilities.

ii. Increase the understanding of broad principles of processes of gene expression and control that may be generalized across species. This research should include studies under a variety of environmental conditions.

iii. Carry out the engineering research of scale-up such that a wide variety of novel products can be economically produced by a range of genetically engineered microorganisms to replace or augment currently expensive materials. Products based upon multiple genes for their synthesis may well necessitate use of biologically organized structures (e.g. orientation of a pathway on a membrane).

iv. Develop cell culture reactors.

v. Develop more cost-effective biological routes for energy sources (e.g., biomass, hydrogen, direct fuel cells) to replace partially petroleum feedstocks.

vi. Incorporate the requirements of scale-up in basic genetic studies of populations of single and mixed species.

b. Surface Interactions of Materials and Biological Systems:

i. Improve the understanding of the behavior of polymers at surfaces (interfaces), including absorption, adhesion, permeability, and ordered crystallization.

c. Structure, Function, Design and Synthesis of Biological Macromolecules:

i. Design protein and enzyme structures at the three-dimensional level.

ii. Design enzyme active sites.

iii. Develop artificial catalysts.

iv. Design macromolecules for detoxification of materials.

v. Incorporate into materials such features of biological systems as complex organization, flexibility, and adaptability; this will depend upon design based on understanding at the whole organism as well as at the molecular level.

D. General Conclusions

The following observations are relevant to the implementation of the research agenda suggested in the previous two sections.

1. A greater understanding of molecular structures is essential for the contributions of biotechnology to progress in the materials sciences. This understanding was also emphasized in the biomolecular electronics workshop.

2. Valuable options for design of products will be lost if all attention is centered on the molecular level; evolution's alternatives should be weighed as models for design through investigations at the organ, organism, and population levels. It is important that research be ongoing at several levels simultaneously, so that integration is possible.

3. Flexibility, adaptability, and complexities of organization are characteristics of biological systems that are long term design goals and deserve basic research investment.

4. Engineers will find exciting new problems in tailoring fermentation and cell culture techniques to large scale processes, and biologists will discover intriguing puzzles by adding engineering constraints as a new variable in their problem solving.

## IV. MICROECOLOGY

The purpose of this workshop was to explore development of multidisciplinary approaches for successful research in the merging of biotechnology and microecology. As used throughout this report, microecology refers to the study of small areas (less than 10 $km^2$) of the marine environment. The approach to this workshop was different from that of the two previous workshops.

The planning committee felt that the areas of study arising from the merging of biotechnology with microecology were at such an early stage of development that it was better to build the agenda around ideas suggested at the workshop rather than around predetermined categories. Therefore, the participants of this workshop spent most of the first day presenting their individual work and discussing questions and proposals stimulated by these studies. That evening the planning committee formulated general questions, based on the day's discussions, for consideration during the next day. The suggestions of research opportunities deserving attention arose in response to these questions, and are described below.

The proposed agenda and some basic initial questions which were distributed to all participants before the workshop are included in Appendix C. Curricula vitarum of all workshop participants are included in Appendix D.

### A. Research Opportunities

Question 1: Biological Consumption of Gases. Nitrogen-fixing blue-green algae (Cyanobacteria) or other marine organisms have the capability to utilize hydrogen, carbon dioxide and carbon monoxide. It may be that these capabilities can be used to help the Navy solve its problems with these substances in submarines. What research would need to be done to make this feasible?

Hydrogen and perhaps other gases are produced in unwanted concentrations aboard submarines. Emission of molecular hydrogen allows submarines to be detected. An objective of a designed system would be to reduce effluent gas concentrations to undetectable levels. Some potentially feasible systems for the consumption of molecular hydrogen may be constructed from assemblages of microorganisms or assemblages of biocatalysts extracted from microbes, plants and animals. Critical basic problems emerge on considering these possibilities.

When the needs are considered in toto, immobilized biocatalysts appear most promising. Some possibilities are ferric heme proteins and enzymes that catalyze the conversion of the hazardous gases to nonhazardous or, better yet, useful substances. For example, ferric heme proteins catalyze the oxidation of carbon monoxide to carbon dioxide. The ferric ion, which is reduced during this process, can be easily re-oxidized by inorganic means. The carbon dioxide concentrated by this process, along with that generated by crew respiration could, in turn, be used to produce formic acid if the enzyme formate dehydrogenase could be made to catalyze the combination of molecular hydrogen and carbon dioxide to form formic acid. This two-catalyst system is one possibility; others may exist, but basic research is needed to discover them. Basic research is also needed to determine whether such systems can be made to perform as desired.

Pure and mixed cultures of microorganisms offer potentially useful gas-scrubbing capabilities. Of particular promise are chemoautotrophic anaerobic bacteria and photoautotrophic blue-green algae (Cyanobacteria). In contrast to the blue-green algae, chemoautotrophic anaerobes such as Hydroxydomonas and Carboxydomonas can convert molecular hydrogen, carbon monoxide, and carbon dioxide into nonhazardous substances, without the secondary problems of toxic by-products or exogenous energy consumption. As with the biocatalysts, basic research is needed, first, to identify microorganisms that can carry out the conversions and, second, to learn how to manipulate them to accomplish the conversions in a cost-effective manner.

Question 2: Chemoreception. Microorganisms have very sensitive systems for the detection of the presence of both nutrients and inhibitors, and they appear to be able to do this both qualitatively and quantitatively. Understanding the mechanisms of chemoreceptor processes would yield useful information for the entire topic of biosensors and biological components of detection systems. What do we need to learn in order to understand enough about the mechanisms to make them useful?

The consensus was clear: basic research is necessary for more complete understanding of the mechanisms underlying chemoreceptions. Previous work on chemical communication has tended to center on large organisms (such as insects) rather than microorganisms. Microorganisms may present quite different functional routes to chemoreception. Furthermore, although a fair amount of work has been done on chemical compounds that may trigger chemotaxis in organisms, the process by which the response of those organisms occurs is relatively unexplored. Particularly important is the molecular basis for recognition of signals and the molecular/physiological basis for avoidance of or attraction toward those signals.

Questions requiring further research include the following:

What are the receptor sites in marine microorganisms? How do they function? To what kinds of signals do receptors respond? Possible signals include chemical compounds, trace elements, currents, light, electromagnetic fields and gradients of various kinds. Studies are needed to determine whether there are specific sensors for each stimulus or whether one receptor can function in many different ways to determine the presence of a range of stimuli. Both similarities and differences among receptors for different sorts of signals may be informative; again, mechanisms must be understood at the molecular level.

How are signals transformed into action or response? How are receptor proteins, for example, "connected" to phenomena of flagellar motility? It might be that avoidance or attraction in chemotaxis is in some ways comparable to a binary "choice", but one which could involve integrated multiple levels of both detection and response. If so, this could have significant implications for detection devices. Along these lines, the kinetics of chemoreception and photoreception responses also deserve study. As a long-term possibility, it may be that chemotaxis in microorganisms may prove important as an interface with biomolecular electronics. Biological systems might actually form the detection part of a sensor, interfacing with a microelectronics component. Alternatively, once biological mechanisms are thoroughly understood, they may be used as a model from which improved solid state detection devices can be devised.

What is the genetic basis for chemoreception? Although current work on chemoreceptor mutants is very pertinent, few investigators are addressing this problem. Once the molecular basis of chemoreception is better understood, it will be possible to address more directly the genetic basis for reception and for response. Genetic engineering might thus be a fruitful area of research in the long term. It may become feasible to identify, clone and transfer genes that are responsible for selectivity of reception to particular signals of interest, or for amplification of response to particular signals.

Finally, the study of chemoreception will underlie nearly all areas to be explored in microecology; film formation and the settling of organisms on surfaces are just two examples among many. Learning about the actual mechanisms with which organisms detect and respond to change will prove fundamental to directed manipulation and use of those inherent biological

capabilities. Development of sensitive, selective sensors may depend upon understanding of how organisms successfully discriminate among the many signals with which they are constantly bombarded in the "noisy" ocean environment.

Question 3: Organisms and Interfaces. The interaction of organisms with interfaces in the marine environment is an important area of study. Both the influence of surfaces or interfaces on the activity of organisms and the impact of organisms on interfaces are of interest. What research needs to be done to define the mechanisms of interaction between organisms and interfaces? How could this information be applied to understanding of microecology and the preservation of surfaces?

The Navy needs to maintain interest and support in the area of biological growth at interfaces and on surfaces. The consensus of the meeting was that an understanding of interfaces and the physical/chemical/biological processes that go on at interfaces in marine systems is tremendously important, and may lead to the ability to use processes productively. Surfaces and interfaces are the sites of active biological processes and often are the dominant influence on the entire system. The sense of the meeting was that information on biological processes could be more helpful in controlling biological growth on surfaces than traditional studies on biofouling or use of inhibitors. The participants question the assumption that the only way to control biological growth on surfaces is through the use of killing agents.

Questions that could be productively explored include:

- What are the basic mechanisms of attraction and initial attachment to surfaces?
- Is binding to surfaces chemical, electronic or physical?
- How does the nature of the surface (nutrient versus non-nutrient, absorptive versus non-absorptive) affect colonization and attachment?
- Are the metabolic processes of organisms at interfaces different in nature or extent from those of free living organisms?
- Can information about biofilms be interfaced with biotechnology processes to produce films with useful properties ("Eco Coats")?
- Can colonization of surfaces by specific organisms or communities that would prevent or retard subsequent growth be encouraged?

- What are the similarities and differences between surface processses and those that occur at other interfaces (i.e. air/water, surface films, etc.)?

- What is the nature of reversible and irreversible binding to surfaces, and what regulates the equilibrium?

The most obvious and direct application of this information is in the control of biological growth on both fixed and mobile surfaces in the marine environment. Once processes and mechanisms have been defined, biotechnology should facilitate the use of natural processes for the prevention or control of biofouling.

Question 4: Products of Organisms. Drag reduction polymers, glycerols, cellulases and other natural products were discussed. Before employing the techniques of biotechnology, what do we need to know about products of organisms in the marine environment?

The group was aware of several examples of directly or potentially useful products that result from the metabolic activity of marine organisms. These include glycerol, cellulases, metal siderophores (for trapping metal nutrients), and drag reducing biopolymers. Many other potentially useful materials are probably produced, but no comprehensive investigation of this area has been conducted. Research into cataloging and characterizing these products might yield useful materials. Both industry and academia are developing extensive background and technology for translating unique/useful biological products into industrial/commercial scale production through genetic engineering and strain selection programs. The potential products could be useful for commercial, medical, scientific and military applications.

Clear connections between this topic and the issues covered by the Materials Sciences Workshop were perceived by the group.

Question 5: Definition of Microecology. A limiting factor underlying adequate definition of how biotechnology can be employed is the lack of understanding of the microecology of the ocean. What do we need to know before we can approach a good definition of microecology?

There was general agreement that biotechnology and microecology are fields that are mutually compatible. Biotechnology can definitely provide an approach to a better understanding of what microecology is and how it can be used. In addition, microecology is an area where present knowledge, although scant, is sufficient to suggest further areas of basic research, as well as applications and "products". In the latter case drag reduction

polymers, biofilm coatings, sensors and atmospheric scrubbers are examples. In the realm of basic research, it was clear that the field of microecology is badly in need of a major infusion of funding. The data base in "mechanisms" and "processes" is remarkably small. Relatively little need exists, at this point, for the development of new equipment or procedures to gather the data for microecology. Using current capabilities, including the new tools of biotechnology, extensive basic research must be conducted if the vast potential of microecology is to be tapped successfully.

## B. Research Recommendations

Specific research recommendations are listed below according to time frame and subject area. The definition of "near term," "middle term," and "long term" are the same as those used in the two previous chapters.

### 1. Near Term Research Recommendations

a. Biological Consumption of Gases:

i. Identify biocatalysts and marine microorganisms with catalytic properties of interest. Examine performance of ferric heme proteins and other enzymes in the catalysis of reactions of molecular hydrogen and other gases.

ii. Determine the hydrogen metabolism of the blue-green algae and chemoautotrophic bacteria, and the mechanisms regulating its control.

b. Chemoreception:

i. Identify compounds and phenomena that act as signals to stimulate chemotaxis in marine microorganisms.

ii. Identify receptor sites in marine microorganisms.

iii. Determine the specificity and sensitivity limits of chemoreception.

c. Organisms and Interfaces:

i. Investigate biofilms.

ii. Compare metabolic processes of free-living organisms and organisms at interfaces.

iii. Study the colonization of surfaces by a variety of organisms; identify characteristics of various surfaces that stimulate or inhibit colonization.

d. Products of Organisms:

i. Catalogue and characterize natural products of marine organisms that might yield useful materials.

e. Definition of Microecology:

i. Identify useful products and capabilities arising in biological systems in the marine environment, such as biofilm coatings, drag reduction polymers, and detection "devices".

2. Middle Term Research Recommendations

a. Biological Consumption of Gases:

i. Investigate comparative enzymology and engineering properties of candidate organisms, including catalytic capabilities, kinetics and stability, and manipulability of rates and yields.

ii. Compare the microbial physiology, reaction mechanisms, and population dynamics of chemoautotrophs and photoautotrophs to determine their suitability as effective systems for elimination of unwanted substances.

iii. Define operational mechanisms for the biological consumption of hydrogen.

b. Chemoreception:

i. Investigate the molecular basis of chemoreception and of the function of receptor sites.

ii. Study the molecular basis of transduction of signals and response to physio-chemical signals to determine what connects receptor proteins to flagellar motility.

iii. Determine how organisms discriminate among a multitude of signals in a "noisy" environment.

c. Organisms and Interfaces:

i. Develop synthetic polymers based on responses of microorganisms to interfaces. Increase knowledge of basic mechanisms of attachment of organisms, including metabolism, physical and chemical bonding, influence of nutrients, and differences between organic and inorganic surfaces. This may have ramifications at various interfaces of the ocean environment in a variety of problems, not the least of which is fouling.

ii. Study the nature of reversible and irreversible binding to surfaces.

d. Products of Organisms:

i. Identify and manipulate genes that code for desired natural products of marine organisms.

e. Definition of Microecology:

i. Investigate the mechanisms of chemical and biological processes in the marine environment.

3. Long Term Research Recommendations

a. Biological Consumption of Gases:

i. Use living biological systems involving blue-green algae and chemoautotrophic bacteria to accomplish consumption of hydrogen or other gases.

ii. Elucidate molecular principles of biocatalysis sufficient to allow use of engineered biocatalytic systems for control of gaseous pollutants.

b. Chemoreception:

i. Investigate the genetic basis for chemoreception and chemotaxis, and the genetic engineering of these capabilities.

ii. Explore the interface of chemotaxis mechanisms with biomolecular electronics in detection devices.

iii. Use chemotaxis mechanisms as a tool with which to study processes and change in the ocean environment.

c. Organisms and Interfaces:

i. Use organisms on surfaces of electronic devices to function as sensors.

ii. Use functions and structures modeled upon interactions of microorganisms with surfaces in sensors.

iii. Engineer microorganisms genetically for desired properties of attachment to particular interfaces.

d. Products of Organisms:

i. Develop commercial, medical, scientific, and military applications of products initially derived from marine organisms and then genetically engineered. The objectives of the Materials Sciences Workshop may well be pertinent here.

e. Definition of Microecology:

i. Continue basic research on mechanisms and processes that constitute the complex ecology of the ocean.

ii. Increase the understanding of what regulates the diversity of organisms present in the ocean. This would include identification of numbers and types of organisms present, as well as knowledge of their metabolic processes and products -- generally, the response of microorganisms to the known variables in physicochemical ocean parameters such as light, pressure, or temperature.

iii. Elucidate how organisms function: with techniques now on hand, including the new molecular tools, it should prove possible to move from previous monitorings of distribution of organisms to such knowledge. Regulation of processes will be of key importance.

## C. General Conclusions

The following observations are relevant to the implementation of the research agenda suggested in the previous two sections.

1. In all the areas discussed, the ability to use organisms or products, either as they are or through genetic manipulation, is limited by what is known about the marine environment. Countless desirable possibilities certainly exist, but basic research is needed in order to learn what is out there.

2. Based on the workshop, several areas for research can be identified that are likely to be fruitful in the relatively near future. These include interactions of organisms with interfaces, chemoreception, and biologically-derived products or materials.

3. In the aggregate, the universities and marine institutes of North Carolina possess significant strength in the marine sciences, with a critical mass and diversity of marine scientists. Among these scientists and others at the workshop there appeared to be a heightened awareness of the exciting potential for multidisciplinary collaboration that is provided by this aggregate strength.

## V. REFLECTIONS

### A. Common Research Themes

Some areas of research emerged as central to the future development of all three fields considered by the workshops. They are:

1. Properties of Surfaces and Interfaces. The biological, chemical, and physical properties of surfaces and interfaces are poorly understood, yet are of extreme importance to advances in biomolecular electronics, materials sciences, and microecology. More needs to be known about the mechanisms by which orgranisms recognize, adhere to, penetrate and damage surfaces. The problems associated with surface and interfacial properties in conventional electronic devices are made more difficult when considering interfaces between biological systems and inorganic materials. Resolution of such problems is necessary if "hybrid" systems are to be developed to function as sensors and as switching and storage devices. The application of specialized instrumentation and analytical techniques hold promise for improving the understanding of surfaces and interfaces.

2. Molecular Structures and Production of New Materials. The design, production and utilization of bio-organic molecules pervaded the discussion in all three workshops. A greater understanding of molecular structures, of structure-function relationships in macromolecules, of protein active sites, and of gene expression is necessary to meet the specific needs in each of the three areas.

3. Understanding Biological Systems. Whether designing new electronic devices, a robot, or a new glue, the workshop participants emphasized the valuable insights that can come from the biological systems that perform similar functions. It is necessary, therefore, that research at the organ, organism, population, and system levels be conducted at the same time that increased efforts are underway to understand molecular processes.

4. Multidisciplinary Collaboration. Perhaps the clearest and loudest common message emerging from the workshops was the statement that multidisciplinary research groups are required to advance the frontiers of knowledge in all areas. How this is best achieved is less clear, but some notions based on the workshop discussions are provided later in this chapter.

It was clear that within all three areas more fundamental knowledge is required before biotechnology can make a sizeable impact. It was noted, however, that among the three areas there are large differences in the degree to

which the problems are defined, in the state of current knowledge, and in the extent of the understanding about future directions and opportunities. The areas of Biomolecular Electronics and Materials Sciences seemed to be fairly well defined with some definite avenues recommended for further research. The area of Microecology, on the other hand, was much less well defined with a consequently broader set of suggestions about where research may lead. These differences were reflected in the formats of the workshops themselves and also are evident in the discussions of the training and equipment needs that follow.

## B. Training

While the purpose of the workshops was to focus on research needs and opportunities, it was inevitable that the topics of training and scientific equipment would be considered. The participants felt strongly that a few points, stated briefly below, warranted inclusion in the workshop report.

The consensus of the Biomolecular Electronics group was that appropriate training -- both for practicing and future scientists and engineers -- will be critical to the future of biomolecular electronics.

Problems peculiar to education in an inherently interdisciplinary area were discussed; for example, how can a student being trained to become a biologist learn sufficient physics, chemistry, and mathematics to conduct research in biomolecular electronics? Most participants felt that students must be solidly trained in their particular disciplines and that broadening activities, including interdisciplinary conferences, seminar series and postdoctoral research, must be included in their education. Further exploration of such activities is necessary.

Sufficient funding is needed to attract students of high caliber to this multifaceted field, if Biomolecular Electronics is to fulfill its promise in the future. Special mention was made of the critical importance of training grants to provide support for graduate students during their first two years of graduate study. It is during this period that students obtain the multidisciplinary perspectives and technical knowledge that make them productive participants in a research program. Once they are able to become part of a research program, their support can be justified as an item in the total costs of carrying out the research.

The need was expressed for the group to meet on a continuing basis to address questions of education. The mix of industries and universities in the Research Triangle area provides an opportunity for unique, high-caliber training programs that will be considered in more detail at these subsequent meetings.

The participants in the Materials Sciences Workshop also felt strongly that training is of critical importance to the future of this field. The retraining of currently active professional scientists and engineers will enable the field to move forward. This may occur to some extent formally, as through technique workshops, but it may also arise through experience with collaborative efforts. Training for the next generation of scientists and engineers may be best addressed not through a proliferation of new degrees, but through augmentation of solid grounding in particular disciplines. Inderdisciplinary courses and accessibility to offerings in widely divergent departments could help students to attain a breadth of understanding in addition to background in their own discipline. Attraction of top quality people to the field -- both as students and as senior professionals -- will help to build upon the current critical

mass, but will require significant financial support.

In discussing the future of Microecology and Biotechnology, training was referred to as the "glue" which could hold together an extended multidisciplinary community. Even though they would be based primarily in one field, graduate students and postdoctoral fellows could serve to "connect" different laboratories, conveying information and techniques and sharing alternative perspectives. Through them, established researchers could grow to appreciate others' activities, and multidisciplinary collaboration in research would be stimulated. The meeting was adjourned with the assurance that with the help of the North Carolina Biotechnology Center the group would continue to meet to consider steps along these lines as well as others. Some of the ingredients necessary to achieve this goal are training program funds, a person to exert strong leadership, and regular opportunities for the group to meet and exchange ideas and progress reports.

C. Equipment

Access to state of the art equipment arose repeatedly as a concern of those attending the Biomolecular Electronics Workshop. Although initial opinions varied as to the type of equipment needed and the extent to which equipment-sharing would be feasible, the consensus was a definite desire to meet further to discuss the identification of common equipment needs. The Microelectronics Center of North Carolina is a pertinent example of a centralized facility for sharing highly expensive equipment. That Center can serve to complement equipment located in the individual universities. Furthermore, it may be possible to add equipment to that Center for use by North Carolina scientists and engineers

involved in Biomolecular Electronics. A high likelihood that common equipment needs exist was indicated by the extent to which research techniques described during the sessions addressed multiple interests. Several collaborations were initiated on the basis of complementarity of techniques, discovered when a researcher in one field heard, perhaps for the first time, about the capabilities of techniques used as a matter of course in another field.

The common equipment need most discussed by participants of the Material Sciences Workshop was a pilot-scale fermentation facility with equipment available for training and research. The construction of such a facility was perceived as beneficial to both academia and industry in solving questions involved in the scaling up of genetically engineered processes. Furthermore, it was felt that the presence of such a facility would serve as the focal point for engineers and biologists to meet and exchange research problems and interests.

The Microecology group did not seem to perceive a driving need for particular sorts of equipment. Instead, the consensus seemed to be that equipment, techniques, and procedures existed for exciting work; but that an infusion of funding into the actual conduct of such studies should be of highest priority.

## D. Planning and Conducting the Workshops

The growth of interdisciplinary collaboration in research and training is a process. The workshops reported on here were undertaken as one approach to facilitating that process. The obstacles to multidisciplinary research and training have been well-documented. Clearly, three workshops cannot, nor were they intended to, overcome all of these obstacles. They did, however, provide a start on collaboration in Biomolecular Electronics, Materials Sciences, and

Microecology among scientists and engineers in North Carolina. We offer here some reflections on the effectiveness of this approach in the hopes of assisting the North Carolina Biotechnology Center, scientists and engineers in the state, the U. S. Navy, and other interested readers in their further attempts at enhancing the quality and quantity of miltidisciplinary research and training in these three areas as well as in other fields.

The planning of each workshop, led by a small, multidisciplinary group of scientists and engineers, itself served a valuable function. Each member of the planning group came to appreciate the different perspectives of the other members. The definition of the problem areas, the organization of the agenda, and the selection of the participants all reflected this wide spectrum of perspectives. Furthermore, proceeding through the planning process, the outlook of the planning group members was broadened as they discovered additional colleagues in a variety of disciplines with whom they were not usually in contact. The workshop began, therefore, with a strong, multidisciplinary approach built in; in their roles as session and discussion leaders, the planning group members continually reinforced this approach throughout the workshop.

The organizational aspects of the workshop that stimulated multidisciplinary interactions included: technical sessions for sharing research programs and scientific successes and failures; a set of common questions to focus the consideration of research recommendations; plentiful time for informal conversation; a situation long-lived enough to allow the group to develop a sense of camaraderie; and the possibility for reconvening the group or subsets of it for follow-up.

In the view of many of the participating scientists and engineers, the workshop presented a rare opportunity for them to participate in a working scientific meeting with individuals from other disciplines. The results were encouraging. The workshop extended the awareness of the participants to research approaches and techniques with which they were not previously familiar. Participants realized that they had common needs for scientific equipment. The merging of biological and physical science perspectives did in fact result in the conceptualization of new research opportunities. A few specific research collaborations were initiated; on a broader scale, the groups supported the suggestion that they continue to meet.

E. Follow-Up

By the end of the workshops, the participants came to realize that if they can continue to work together significant capabilities exist in North Carolina for pursuing opportunities in each of the three areas. The best way to continue, the participants felt, was to meet in groups organized around areas of common scientific interest. It was recognized that needs for expensive, sophisticated equipment and for a wide range of training capabilities can serve as bases of this common interest, along with particular research problems. Such small-group meetings can nurture the trust and understanding so essential to scientific collaboration.

The participants expressed appreciation for the critical role the North Carolina Biotechnology Center had played in bringing together all the parties involved in planning and conducting the workshop. They requested that the

Center continue to perform these functions in assisting the groups as they seek to stimulate further collaboration in research and training. The Center would serve as a catalytic force in convening the groups and in providing support services, and would not in any way serve as a bureaucratic, superstructure controlling the action. For multi-institutional collaborative projects, the Center might serve as the locus for project administration and coordination, with scientists and engineers from the participating institutions responsible for technical decisions. Indeed, this is precisely the model that has proved so successful in carrying out the workshops. Efforts among various multi-institutional groupings might well necessitate variations on this same general approach. Just as the development of the workshops called for a spirit of flexibility, cooperation, and willingness to experiment, so these next steps in the process are likely to require the same spirit from the North Carolina Biotechnology Center and the scientists, engineers, and institutions involved.

APPENDICES

A. BIOMOLECULAR ELECTRONICS

Agenda
Abstracts
List of Attendees

B. MATERIALS SCIENCES

Agenda
Abstracts
List of Attendees

C. MICROECOLOGY

Agenda
List of Attendees

D. CURRICULA VITARUM

## A. BIOMOLECULAR ELECTRONICS WORKSHOP SEPTEMBER 15-16

AGENDA

September 15

| | |
|---|---|
| 7:30 a.m. | BREAKFAST |
| 8:00 a.m. | Introductory Remarks.....Richard Johnson, presiding |
| 8:10 a.m. | Biomolecular Electronics and North Carolina.....Quentin Lindsey |
| 8:20 a.m. | Biomolecular Electronics and the U. S. Navy.....Robert Newburgh |
| 8:50 a.m. | Workshop Overview, Objectives, Logistics.....Laura R. Meagher |
| | SESSION I. ORGANIC ANALOGS TO SEMICONDUCTOR DEVICES.....Klaus Bachmann, Chair |
| 9:00 a.m. | Design and Synthesis of Novel New Materials For Conductors, Semi-Conductors, and Superconductors.....Robert Bereman |
| 9:30 a.m. | Spatially Isolated Orbitals-- Intramolecular Exciton and Electron Hopping.....Keith DeArmond |
| 10:00 a.m. | Biological Substrates for Micro-electronic Applications.....Steve Quint |
| 10:30 a.m. | Communication Networks in Cardiac Tissue.....Jack Buchanan |
| 11:00 a.m. | Conclusions (Research Recommendations) |
| 12:15 p.m. | LUNCH |

September 15 (Continued)

SESSION II. INTERACTION OF RADIATION WITH ORGANIC MATERIALS AND BIO-CHEMICALLY ACTIVE SURFACES.....Carl Bumgardner, Chair

| | |
|---|---|
| 1:30 p.m. | Auger, ESCA and XAFS analyses of solids with Applications to Biochemical Reactions.....Michael Paesler |
| 2:00 p.m. | Computer Analysis of Micro-Video Images.....James A. Knopp |
| 2:30 p.m. | Ion Electron and Photon Interactions with Materials.....Thomas Mayer |
| 3:00 p.m. | Organic Detectors.....Frank DiBianca |
| 3:30 p.m. | BREAK |
| 3:45 p.m. | Conclusions (Research Recommendations) |
| 6:00 p.m. | DINNER |
| 7:15 p.m. | Frontiers of Microelectronics.....Don Beilman |
| 8:00 p.m. | Discussion, Educational Programs.....Don Phillips, Chair |

September 16

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| 7:30 a.m. | BREAKFAST |
| 8:00 a.m. | Advances in Molecular Micro-Electronics.....Forrest Carter |
| | SESSION III. INTEGRATION OF BIO-MOLECULAR SYSTEMS WITH MICROELECTRONIC DEVICES.....Richard Johnson, Chair |
| 8:45 a.m. | Photon-Induced Charge-Transfer, Corrosion and Passivation at Semi-conductor/Electrolyte Interfaces.....Klaus Bachmann |
| 9:15 a.m. | Modification and Characterization of Inorganic Surfaces for Immobilization of Biochemicals.....Harold Swaisgood |
| 9:45 a.m. | Electrochemistry of Bioorganic Molecules Covalently Attached to Non-metallic Microelectrodes.....C. W. Anderson |
| 10:15 a.m. | Electron Imaging and Analysis of Biological Specimens and Micro-circuits.....Jacob Hanker |
| 10:45 a.m. | Conclusions (Research Recommendations) |
| 12:00 p.m. | LUNCH |

September 16 (Continued)

SESSION IV. BIOMOLECULAR CIRCUITS AND SYSTEMS.....Jacob Hanker, Chair

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|---|---|
| 1:00 p.m. | Characterization of Sub-Micron Device Structures.....George Rozgonyi |
| 1:30 p.m. | Biological Electron Transfer Systems....James Siedow |
| 2:00 p.m. | Limits of Microminiaturization of Electronic Circuits.....Michael A. Littlejohn |
| 2:30 p.m. | Stability Considerations in 3-Dimensional Biologically Based Structures.....Richard Johnson |
| 3:00 p.m. | Conclusions (Research Recommendations) |
| 3:30 p.m. | BREAK |
| 3:45 p.m. | Overview Discussion of Workshop conclusions, led by Bachmann, Bumgardner, Hanker, and Johnson |
| 5:00 p.m. | Conference Ends |

# ABSTRACTS

## Design and Synthesis of Novel New Materials

## For Conductors, Semiconductors, and Superconductors

Robert D. Bereman
Professor
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Raleigh, North Carolina 27650

Our laboratory has recently been involved in both an extensive synthetic program designed to develop active mimics of metalloenzymes and an extensive synthetic program centering on new organosulfur ligands, and their solid state conductivity properties. As such we are in an ideal position to consider the ultimate "mating" ob biotechnology and microelectronics in biomolecular electronics. The talk will present a brief review of the status of materials called "organic metals" as well as attempt to show the limitations of current programs. A review of a work on organosulfur coordination complexes will also be presented and analogies will be made to the work on tetrathiofulvalene. The organosulfur ligands have been designed to not only alter the electronic properties of a coordinated metal center but also, through the electron delocalization present in the ligand, to resemble aspects of "organic" metals. The current status of a program as well as the ultimate objectives will be presented. Specific attention will be given the role of the synthetic chemist in biotechnology.

ABSTRACT

Spatially Isolated Orbitals – Intramolecular Exciton and Electron Hopping

By M. Keith DeArmond, Kenneth W. Hanck and Dennis W. Wertz

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Systematic studies of the relaxation of excited electronic states of transition metal complexes have utilized absorption and emission spectra, decay lifetimes, quantum yields, and most recently, photoselection spectra to indicate a complicated and varied relaxation pattern. The orbital parentage of the lowest lying excited states for these nominal $d^6$ complexes of Ru(II), Os(II), Rh(III), and Ir(III) can vary from metal localized (dd*) to charge transfer ($d\pi^*$ or $\pi d^*$) to ligand localized ($\pi\pi^*$). This wide range of possibililties results in a unique photophysical phenomenon: multiple state emission which indicates that under certain conditions vibronic coupling cannot occur between low lying excited states.

Two types of multiple state emission have been identified for these $d^6$ complexes and for some $d^8$ and $d^{10}$ complexes. The first type "distinct orbital origin", i.e., dd* or $d\pi^*$, was initially reported by Watts and coworkers $[Ir(bpy)_2Cl_2]^+$. The second, "spatially isolated emission", was first observed for the $[Rh(bpy)_2(phen)]^{3+}$ and $[Rh(bpy)(phen)_2]^{3+}$ complexes where the emission originates from both the bipyridine and phenanthroline rings.

The occurrence of the "spatially isolated" or "single chelate ring" emission suggested that the parent tris complexes ($RhL_3^3$, L = bpy or phen) might also exhibit single ring emission. The postulaton of such single ring orbitals for the $[Ru(bpy)_3]^{2+}$ ion. The ESR indicates that the additional electron reduced the bipyridine ligand so that the ion could better be represented as $[Ru^{II}(bpy)_2(bpy^-)]^+$. The first bona fide experimental evidence for single ring orbitals in the emitting state of $[Ru(bpy)_3]^{2+}$ came from the excited state resonance Raman work of Woodruff and coworkers which indicated that, on a vibrational time scale, the electron is localized on one ring. Our photoselection studies have verified these results and extended the time scale of localization to the microsecond domain at 77K. This result is quite unexpected since the emitting state has been characterized as a $d\pi^*$ type and delocalization occurring through the $Ru^{2+}$ metal ion is expected.

Thus, data indicates the presence of localized orbitals (optical and redox) for these imine chelate complexes of $d^6$ metal ions. The experimental evidence for this localization will be described and some description (origin, height) of the barrier to the exciton and electron hopping will be presented.

# BIOLOGICAL SUBSTRATES FOR MICROELECTRONIC APPLICATIONS

Stephen R. Quint, University of North Carolina at Chapel Hill

The excitable cell membrane is the substrate for current amplification in excitable cells, in direct analogy to the semiconducting substrate of the transistor. In examining this electronic-bioelectric analog we have demonstrated, in our previous research, the functional and theoretical isomorphism between these devices when a transistor is substituted for the voltage dependent sodium and potassium conductances in the Hodgkin-Huxley electrical model of the excitable membrane. With all other elements of their model intact an avalanche circuit, with monostable operation and negative resistance characteristics, is obtained which demonstrates most of the basic excitable membrane characteristics. These include threshold activation, large conductance changes due to multiplication, initiation of an output pulse by reduction in voltage (depolarization), overshoot, and the anodal break response. With the addition of a passive R-C section to simulate an axon, the circuit exhibits temporal summation of subthreshold inputs and after-positivity following activation, with an associated refractory period. The theory of operation of semiconductors is well understood. However, this is not the case with channel properties of the excitable membrane. Synthetic bilayer lipid membranes can be constructed, into which ionic channels may be introduced by "doping" methods. These channels may be made to demonstrate many of the voltage dependent conductance properties necessary for the electrical excitability of living membranes (including negative resistance regions of operation) without exhibiting "all-or-none" type excitability. Current techniques avalable for studying channel properties in both excitable and synthetic bilayar lipid membranes, including voltage clamping, patch clamping, and noise analysis, may reasonably be expected to give additional knowledge in the bioelectric mechanism of membrane excitability, with possible applications to microelectronics. Additionally, it is likely that, by altering the excitable membrane "bias potentials" using the patch clamp technique, a biological analogy of transistor configuration, other than the avalanche type, may be configured, again with implications for microelectronic design.

# COMMUNICATION NETWORKS IN CARDIAC TISSUE

Jack Buchanan, University of North Carolina at Chapel Hill

The conducting system of the heart, from the point of view of a systems engineer, performs a well described function with a high degree of reliability and built in redundancy and, unlike the nervous system, with no need for chemical transmitters. In addition, although it can function with no input from the central nervous system, its activity is modulated by the nervous system by mechanisms which are just now being discovered. The heart contains a number of potential oscillators to serve as a clock for the intrinsic heart rate, one of which predominates at a given time. In addition there are cells throughout the conduction system of the heart which have a normally suppressed capability to take over this role if the normal conduction pathways are cut. Although there is thought to be a single mechanism for the electrical coupling of impulses from one cell to the next, the conduction velocity in various portions of the heart varies over a 200 fold range in order to properly synchronize the contraction of the heart. The propagation pathways of the heart can be analyzed using standard transmission line theory, although once a section of membrane is excited it depolarizes in a highly nonlinear fashion. We have measured a number of the passive (transmission line) parameters in guinea pig ventricular muscle and are combining this information with mathematical models for nonlinear membrane depolarization in order to develop a model for the propagated response. These techniques and results may have some usefullness with respect to present VLSI design problems and an analysis of desirable and undesirable characteristics of impulse propagation along biological membranes is of importance to future biomolecular circuit design. Of additional interest to the circuit designer is the recent discovery of cells of the heart which under certain conditions have two stable resting membrane potentials and can be trigsered from one state to the other. An understanding of this process may have important implications for future biomolecular circuit design.

# ESCA, Auger and EXAFS Studies of Chemical Environments in Biological Systems

M.A. Paesler

Department of Physics

North Carolina State University

We discuss the basic physics of three analytical techniques used in the Department of Physics at North Carolina State Universiyt. Photoemission investigations of local bonding environments are carried out on an ESCA (Electron Spectroscopy for Chemical Analysis) Spectrometer while Auger spectroscopy is used to determine chemical composition of samples. In a program administered through the Department of Physics, EXAFS (Extended X-ray Absorption Fine Sturcture) studies are carried out at the National Synchrotron Light Source at Brookhaven National Laboratory. As an example of the use of these techniques, a study of iron-protein interactions is discussed.

# "Computer Analysis of Micro-Video Images"

James A. Knopp
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Our laboratory has been involved with the measurement and analysis of changes in the surface properties of biological preparations. Specifically, we have been infusing our samples with fluorescent molecules which respond to the presence of oxygen by a decrease in both the intensity and the decay time of fluorescence. This technique provides the only non-invasive measurement of oxygen in biological samples. In order to achieve spatial as well as temporal resolution, I have developed instrumentation which will digitize and compare frame by frame individual picture elements, i.e. pixels. The comparisons are performed using microcomputers and the results are displayed as color-enhanced video images and as hard copy plots. Such procedure permits the following types of analysis: 1) Movement artifacts in the surface, either locally or general. This procedure permits the rapid realignment of surfaces to a previous reference frame. 2) Quantitative changes in local surface detail. Both the location and the extent of change can be documented during the modification of surfaces. 3) Percentage changes. Through direct subtraction and ratio calculations, the detection of significant changes can be determined in the presence of noisy images. These procedures and instrumentation are not limited to our measurements but can be applied to any image from EM pictures to satellite photos. Our major contribution is in the quantitative analysis to give image ratios. Further immediate progress should be in the direction of developing techniques for real time analysis and higher resolution. It is anticipated that these techniques will be needed in the construction of surfaces and may provide the ultimate I/O device for photo-active surfaces.

# ION ELECTRON AND PHOTON INTERACTIONS WITH MATERIALS

T. M. Mayer
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Interactions of energetic particles with surfaces of materials can bring about a variety of physical and chemical modifications of the material. We will concentrate on energy deposition processes in surfaces and selective, chemical alterations of surfaces by energetic particle impact. A number of examples in thin film growth and etching of semiconductor materials will be presented.

Degradation and modification of organic materials by particle impact has been studied primarily out of interest in microlithography. Energy deposition, degradation and crosslinking processes which permit microstructure definition will be described. Chemical modification of organic and polymer surfaces and deposition of thin polymer films and possible applications of these processes will also be introduced.

# ORGANIC DETECTORS

Frank A. DiBianca, University of North Carolina at Chapel Hill

Detection devices based on either organic or inorganic materials are widely used in virtually all fields of science and engineering. The subject of organic detectors can be divided into three areas: nonbiological organic detectors, bio-organic detectors and biological detectors.

Nonbiological organic detectors are useful because they often possess properties which may be difficult to find in inorganic detectors with similar functions. For example, organic liquid and plastic scintillators used for detecting ionizing radiation such as x-rays and ions have faster time responses (nanosecs) than most inorganic scintillators (microsecs) and lower afterglow and hysteresis as well. By loading such substances with high atomic number organometallic compounds one obtains considerably higher x-ray stopping power allowing their use in medical computed tomography and digital radiography detectors (F.A. DiBianca and D.A. Cusano, US Patent 4,262,202). A further example of a nonbiological organic detector is that of a class of organometallic semiconductors which change chemical state upon exposure to electromagnetic fields or light. It has been shown (R.S. Potember et al.) that compounds such as Ag-TCNQ (silver-tetracyanoquinodimethane) will undergo oxidation-reduction reactions which release metallic silver and thereby increase conductivity in the presence of electric fields or laser light. These chemical and electrical changes are reversed by the action of higher powered carbon dioxide lasers suggesting possible use in fast optoelectronic switches and erasible optical memories.

The second class to be considered, that of bio-organic detectors, includes those based on biological macromolecules such as protein molecules. Detectors of this type may make use of genetic coding to determine the electrical, chemical, optical or other input and output responses. Such detectors could therefore be very selective in response to input stimuli and could produce quite specific output responses.

The third class of organic detectors, i.e., biological detectors, includes those based on cultured living cells and possibly even living micro-organisms. Obvious general advantages would be the capability of reproduction, conditioning (learning?) and mutation (genetic modification), the latter allowing possibly beneficial new attributes or functions. Further modification of such biological materials by attaching metal ions at specific sites or applying conducting metallic coatings (J.S. Hanker et al.) could allow studies of possible uses as electrical or electronic detectors. However questions arise as to their viability under the required environmental conditions as well as under exposure to potentially high levels of input energy. The answers to these and other questions await further research in the field of organic detectors.

# Photon-Induced Charge-Transfer, Corrosion and Passivation at Semiconductor/Electrolyte Interfaces

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Since biological systems achieve the transfer of electronic charges by means of ionic species in aqueous solutions, the utilization of microelectronic and/or optoelectronic devices based on semiconductors as either sensing, signal processing or control elements in a hybrid biochemical-solid state electronics system requires charge-transfer across the interface between a metal contacting the semiconducting element and an electrolyte or direct charge-transfer from the semiconductor into the electrolyte. Optoelectronic circuits that probe biochemical reactions by interactions with light do not need to provide for the exchange of electronic charges, but in this case the reliability of the semiconductor circuit is still affected by interactions with the biochemical environment. Therefore, the understanding of charge-transfer, corrosion and passivation phenomena at semiconductor-electrolyte interfaces is essential for optimizing the design and operation of hydrid biomolecular electronics systems. In this paper the band bending, minority carrier charge-transfer, corrosion and passivation at semiconductor/electrolyte interfaces is reviewed with special emphasis on photon-induced processes that may become of interest in the context of controlling biochemical processes by injection of photogenerated carriers and of combining optoelectronic sensors with biological systems. Most of the experience in the photoelectrochemistry at semiconductor electrodes has been generated in the past by studies aiming at the conversion of solar energy into electrical power or chemical fuels. The most stable and efficient structures discovered to date in this area of application are electrolyte-insulator-semiconductor (EIS) devices where the minority carrier transfer to the redox receptor in the electrolyte occurs by tunneling.[1-5]

# MODIFICATION AND CHARACTERIZATION OF INORGANIC SURFACES FOR IMMOBILIZATION OF BIOCHEMICALS

H. E. Swaisgood
Department of Food Science
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Reaction of the silanol groups of inorganic matrices such as silica or glass with organosilanes provides a convenient method for derivatization of these surfaces. Thus treatment with γ-aminopropyltriethoxysilane yields, on surface polymerization via formation of siloxane bonds, a matrix coated with aminopropyl chains. If desired, this surface of primary amino groups can be converted to a surface of carboxyl groups by treatment with succinic anhydride. Alternatively, the surface can be coated with glycol groups by reaction with γ-glycidoxypropyltrimethoxysilane. Various biological molecules, large or small, can be attached covalently through these functional groups yielding a large variety of possible surface environments. Covalent attachment through carboxyl groups using a water-soluble carbodiimide for activation was examined in detail for a number of biochemicals. Proteins and enzymes can be covalently bound without exposure to the reagent which might impair biological function. Methods were developed for determination of the characteristics of these surfaces by measuring the partition coefficient and specific binding constants for molecules present in the bulk phase. Such measurements demonstrated dramatic effects of surface derivatization on the characteristics of the matrix phase.

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Brown, R.J., Swaisgood, H.E., and Horton, H.R. (1979) *Biochemistry 18*:4901-4906.
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Janolino, V.G. and Swaisgood, H.E. (1982) *Biotechnol. Bioeng. 24*:1069-1080.

# Electrochemistry of Bioorganic Molecules Covalently Attached to Non-metallic Microelectrodes.

C.W.Anderson and D.J.Arnold

High energy reactions such as those found in non-equilibrium plasmas and high temperature gasses allow for the rapid and extensive functionalization of surfaces generally regarded as inert. The surfaces of interest here are carbon; specifically, they are: 8 micron and less diameter carbon fibers and 0.02 micron and less carbon films. By functionalizing the surface of these electrodes, it is possible to covalently attach molecules with some desirable property directly to the electrode surface and use the electrode to either control the surface attached species or respond to it.

Several different types of attachment procedures have been used and several types of molecules have been attached. Those attached species that will be addressed include neurotransmitters (catecolamines), synthetic enzyme model compounds (binuclear Cu cyclodextrin), and functioning proteins (cytochrome c, soy bean lipoxygenase and hemoglobin).

# ELECTRON IMAGING AND ANALYSIS OF BIOLOGICAL SPECIMENS AND MICROCIRCUITS

J.S. Hanker, University of North Carolina; B.L. Giammara, University of Louisville; J.H. McAlear and J.M. Wehrung, EMV Associates

The properties of atoms and molecules which determine X-ray or electron opacity will be reviewed. It will be shown that ordinary density may be more important than atomic number, Z, in determining backscattered electron imaging as well as electron contrast of an element. Chemical reactions developed in our laboratories which result in the selective deposition of electron-opaque and conductive compounds at fixed sites of tissue structural macromolecules, enzymes and antigens will be demonstrated. These reactions are chemical amplification reactions in that repetitive bridging or catalytic polymerization may be utilized to increase the amounts of electron-opaque or conductive metal compound deposited at the sites of the biomolecule.

Studies with newly synthesized highly electron opaque and conductive silver coordination compounds as end products in these reactions will be described.

Complementary light microscopy, transmission electron microscopy, STEM, and scanning electron microscopy (secondary and backscattered electron) studies of biological specimens imaged by these reactions will be shown. Supplementary information from X-ray microanalysis will be included. The potential use of this technology in the fabrication, function and analysis of microcircuits will be inferred.

Preliminary studies of the factors and problems encountered in the adherence of cerebral cortical neurons to resist patterns will be presented.

# Characterization of Sub-Micron Device Structures

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and
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Both the fabrications of microelectronic devices and its underlying materials science, which provides the building blocks for VLSI circuits, require an extensive array of sophisticated characterization tools. This presentation will attempt to give an overview of those instruments which reveal the morphology and microscopic architechture of individual devices, as well as those sub-surface crystal defects which affect device performance. Selected examples of sub-micron diagnostics of molecular beams epitaxy, laser processing and impurity/defect gettering will be discussed.

BIOLOGICAL ELECTRON TRANSFER SYSTEMS, James N. Siedow, Department of Botany, Duke University

Considerable progress has been made in understanding electron transfer through multicentered biological redox systems. Some of the mechanisms associated with electron flow through these systems should provide insights into solutions to problems which will arise in the development of bioelectronic devices. The chloroplast electron transfer chain consists of two photosystems operating in series and the mechanism by which light energy is transformed into chemical energy at the reaction center chlorophylls provides a useful model system for photoactive devices. The use of picosecond optical spectroscopy has allowed a sequencing of the earliest electron transfer events in the photosynthetic reaction centers; while more conventional techniques have been used to elucidate the path of electron flow beyond the reaction centers. Of potential interest to biotechnology is the gating mechanism by which electron flow is switched from a one-electron to a two-electron reaction and back again. The mitochondrial electron transfer chain also demonstrates several interesting features of complex electron transfer systems. These include mechanisms 1) which provide for electron flow both within individual multicentered complexes as well as between separate complexes, 2) for bringing about a four-electron reduction of oxygen to water and 3) for alternating one- and two-electron transfers. Finally, the enzyme xanthine oxidase provides an example of solid state electron transfer between multiple redox centers in a system which is not membrane bound. The above examples provide a limited view of the range of biological electron transfer systems which are available for use in the development of bioelectronic technology.

# CONSTRAINTS AND OPPORTUNITIES FOR VLSI

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## ABSTRACT

Advances in semiconductor technology during the last several years have brought about the present era of very large scale integration (VLSI). These advances have been realized in the area of process technology, including lithography, etching, interconnections, materials, and low-temperature processing. Other important achievements have come about in design automation and packaging and testing. Because of technological innovation in this field, integrated circuits containing over 100,000 devices with 1- to -2 micrometer feature sizes have become a manufacturing reality. Now that VLSI is a reality, there are key questions which face researchers in this field. In particular, it is important to ask where and how far VLSI can be extended.

There are constraints and opportunities for VLSI. In many cases, the constraints are technological. New opportunities will also come because VLSI offers a realm for the exploration of physical phenomena and the development of new physical mechanisms. The university research community will be heavily involved in understanding the constraints and realizing the opportunities. In addition to working on contemporary problems of VLSI, this community must also consider the problems from a long-term perspective, perhaps 5-15 years in the future.

Biotechnology is a technology of the future. There is a strong interconnection between VLSI today and biotechnology in the future. These two communities should begin to work together today so that both might appreciate their future roles in technology.

# STABILITY CONSIDERATIONS IN 3-DIMENSIONAL BIOLOGICALLY BASED STRUCTURES

RICHARD N. JOHNSON, UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

Progress in microelectronics is coupled with the ability to continually place larger numbers of smaller devices on a single chip. The problems associated with very large scale integration (VLSI) and continually smaller device structures are extensive. The separation of device design from system design depends on being able to isolate individual devices, except for desired interconnections. In dense device arrays, line to line capacitance and nearest neighbor interactions begin to dominate the system. Thus, unwanted device or system characteristics may arise. The 3-dimensional structures utilized in living systems have the potential for illustrating solutions to the high density device interaction problem. The mammalian central nervous system (CNS), due to the extreme packing density and high connectivity of cellular structures, can exhibit a variety of unstable behaviors, clinically described as the epilepsies. Beginning in the early 1970s, we conducted a number of basic research studies on stability relationships within the central nervous system, with particular focus on the cerebellar-thalamocortical motor system. In the middle of that decade, chronic electrical stimulation of the cerebellar surface was introduced to treat selected patients with epilepsy and motor disorders. It is very clear from the above studies and the work of numerous other investigators that the mammalian CNS employs significant structures whose sole purpose appears to be to maintain stability in the dense, highly interconnected 3-dimensional networks of the CNS. Information on neural circuit architecture can play a significant role in VLSI circuit design at the molecular dimension level. Current work being conducted on organized neural cell cultures, coupled with theoretical studies on models of neuronal networks, and **in vivo** studies can provide this information. The potential of macromolecular and multicellular systems for bimolecular electronics is great in terms of size reduction, power reduction and with enhanced performance characteristics.

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## B. MATERIALS SCIENCES WORKSHOP SEPTEMBER 30-OCTOBER 1

AGENDA

September 30

| | | |
|---|---|---|
| 8:00 a.m. | Biotechnology and North Carolina..... | Don I. Phillips |
| 8:15 a.m. | Workshop Overview, Objectives, Logistics..... | Laura R. Meagher |

SESSION I

CHEMICAL FEEDSTOCKS, ENERGY, AND PRODUCTION OF CHEMICALS

David E. Guinnup, Chair

| | | |
|---|---|---|
| 8:30 a.m. | Biologically-based Industries: Past and Future ..... | Howard G. Clark |
| 9:00 a.m. | Chemicals for Explosives and Coatings Manufacturing ..... | A. F. Coots |
| 9:30 a.m. | Applications of Tissue Culture Biotechnology ..... | Henry V. Amerson for Ralph L. Mott |
| 10:00 a.m. | BREAK | |
| 10:15.a.m. | Analysis of Multicomponent Systems with Monoclonal Antibodies ..... | Robert E. Johnston |
| 10:45 a.m. | Engineering Practices for Scale-up of Genetically Improved Processes ..... | David E. Guinnup |
| 11:15 a.m. | Conclusions (Research Recommendations) | |
| 12:15 p.m. | LUNCH | |

September 30 (Continued)

SESSION II

STRUCTURE AND FUNCTION OF BIOLOGICAL MACROMOLECULES

Ann Matthysse, Chair

| | | |
|---|---|---|
| 1:30 p.m. | Mechanism of Action of Dehydrogenase Enzymes | John H. Harrison |
| 2:00 p.m. | Chemical Modification of Enzymes as Probes of Active Sites and Macromolecular Structure | H. Robert Horton |
| 2:30 p.m. | Design and Potential Application of Model Enzymes | R. D. Bereman |
| 3:00 p.m. | BREAK | |
| 3:15 p.m. | Polymer Surface Control of the Biological Formation of Carbonates | Miles A. Crenshaw |
| 3:45 p.m. | Metallothionein in Drosophila | Gustavo P. Maroni |
| 4:15 p.m. | Conclusions | |
| 6:00 p.m. | DINNER | |
| 7:15 p.m. | Address; U. S. Navy Representatives | Larry Peebles and Stanley Brown |
| 8:15 p.m. | Discussion: How to Carry on Collaboration Begun in this Workshop | Don Phillips Chair |

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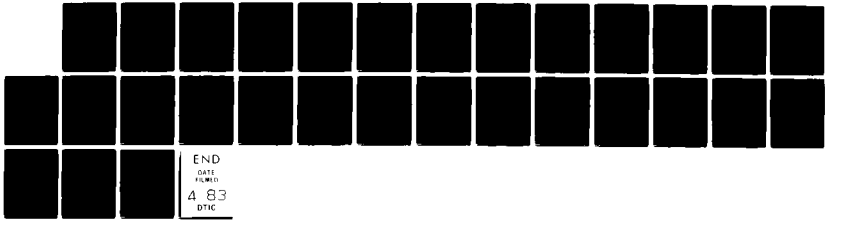

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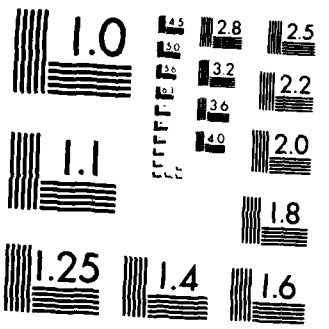


MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS 1963 A

October 1

7:30 a.m. BREAKFAST

SESSION III

SURFACE INTERACTIONS OF MATERIALS AND BIOLOGICAL SYSTEMS

Howard Clark, Chair

8:00 a.m. Adherence of Phytopathogenic Bacteria to Plant Cells ..... Ann G. Matthysse

8:30 a.m. Interactions between Marine Fungi and Wood Borers ..... Jan Kohlmeyer

9:00 a.m. Materials-Related Surface Phenomena ..... C. M. Balik

9:30 a.m. BREAK

9:45 a.m. Immobilized Oxygen Carriers as Biomolecular Gas Extractors and Pumps ..... Joseph Bonaventura

10:15 a.m. The Design of Marine Structures ..... S. A. Wainwright

10:45 a.m. Conclusions

12:00 p.m. LUNCH

October 1 (Continued)

SESSION IV

DESIGN AND SYNTHESIS OF PROTEINS

Ann Matthysse, Chair

| | |
|---|---|
| 1:15 p.m. | Protein Synthesis in Chloroplasts ..... Linda L. Spremulli |
| 1:45 p.m. | Rational Design of Functional Proteins.....Thomas Vanaman |
| 2:15 p.m. | Using Concepts of Protein Topology and Structure Design to Design New Proteins.... Jane S. Richardson |
| 2:45 p.m. | Conclusions (Research Recommendations); Overview Discussion of Workshop Conclusions led by Clark, Matthysse and Overcash. |
| 4:00 p.m. | Conference Ends |

## Biologically-based Industry: Past and Future

Howard G. Clark
Department of Biomedical Engineering
Duke University

### Abstract

Within this century the United States has moved from an economy which was largely dependent on biological sources for materials (excluding metals) through a partial dependence on coals, into an economy which is heavily based on petroleum as a starting material for synthesis of materials. Since the OPEC oil embargo of 1973-1974 there has been an awareness that U.S. petroleum resources are encapable of meeting our national needs for energy and materials synthesis. Foreign petroleum resources are also finite and subject to increasing demands from the rest of the world. Petroleum will be more valuable as a raw material for chemical syntheses than as a source for electric power, but will continue as an energy source for transportation. Hence petroleum as a feedstock for chemicals will persist after its use for electric generators is substantially replaced by other technologies.

The momentum of new science encompassed by the term "molecular biology" will lead to new technologies which will result in new materials. The relative economic advantage of petroleum based industries will also be shifted to make biological based industry more competitive. Thus the present era is probably a nadir in biologically-based industry which predominated the past and will dominate the growth pattern of the future.

New biologically-based industry will not be a return to the past but rather a forward step from the present, i.e. glycerine may be synthesized as a pure compound by bacteria rather than produced by hydrolysis of animal or vegetable fats. Microbial reactions offer low energy, low pollution methods of modifying feed-stocks based on petroleum while catalysts derived from petroleum research may permit economic conversion of fermentation alcohol to gasoline. Cell culture, immobilized enzymes, and immobilized micro-organisms offer new techniques not available to an older fermentation industry. Cell and organelle separation technology which has been developed for medical research offers addtional new methods for synthesis of materials.

There is already an extensive microbiology of petroleum based on studies which were necessitated by bacterial acceleration of corrosion, particularly with high sulfer crudes, and the souring of drilling muds by microbial action. Microbial action has been invaluable in eliminating the pollution characteristics of petrochemical and other industrial waste, but engineering studies of scale-up, control, and waste disposal have lagged behind modern biochemistry in preparing to capitalize on the opportunities which lie ahead.

# CHEMICALS FOR EXPLOSIVES AND COATING MANUFACTURING

A. F. Coots

Types of explosives and propellants will be described, and some of their important characteristics will be discussed. Some problem areas in explosive technology will be discussed, and research areas will be identified.

The components of paint will be discussed. Potential research and development in coating technology will be considered.

# APPLICATIONS OF TISSUE CULTURE BIOTECHNOLOGY

Dr. Ralph L. Mott
Plant Cell & Tissue Culture Lab.
Department of Botany
N. C. State University
Raleigh, North Carolina

An overview of available tissue culture methods will be presented, using laboratory examples. Many of these methods are now used commercially. There are many more potential uses. One set of potential uses lies in the generation, selection and rapid clonal propagation of desirable plant types from among the plant types available in nature. Specialized, superior planting stock can easily be obtained in this way without long-term breeding stabilization programs. A second set of potentials lies in the free cell and organ cultures which permit *in vitro* study of cell and/or tissue physiology, gene expression and even the separate stages in whole plant development. New avenues are thus provided for learning about molecular and cellular interactions in plant responses. Such understanding can be applied to better management and control of useful responses in plant crops. The knowledge can also be applied to production of desirable chemicals directly from cells growing in vat cultures. A third set of potentials arises from the use of the cultures of cells and/or protoplasts (living cells with walls removed) as the experimental materials to which gene splicing technologies can be applied. This artificial genetic manipulation could of course yield new genetic types not otherwise available; eq., entirely new types of plants or vat-cultured cells or common cell types having one or two new capabilities.

As world petroleum supplies for energy and biochemical feedstocks become depleted, the ability to quickly modify plants to suit these new biomass needs could become critically important. The tissue culture methods can provide that ability.

# ANALYSIS OF MULTICOMPONENT SYSTEMS WITH MONOCONAL ANTIBODIES

Robert E. Johnston

## ABSTRACT

Monoclonal antibodies are being used in an investigation of those molecular characteristics of Sindbis virus (SB) which determine its virulence in animals. Monoclonal antibodies are biological reagents which can be selected for their ability to recognize specific domains in proteins and to bind these domains by non-covalent interactions. Such reagents can be used not only to probe molecular differences between the isolated proteins of wild-type SB and avirulent mutants, but also can be used to analyse the interactions between proteins in the virions and the topology of the virion surface. Similar types of analysis using specific monoclonal antibodies can define functional domains in proteins such as enzyme active sites and can be used in the isolation and immobilization of active enzyme preparations.

# Engineering Practices for Scale-Up of Genetically Improved Processes

David E. Guinnup, Chemical Engineering Department
North Carolina State University

The advent of recombinant DNA techniques has led to the genetic modification of many types of bacteria which can be used in the production of industrially-useful compounds. Since these breakthroughs generally occur on a small scale in the laboratory, it becomes important to be able to translate them into large-scale processes in order to realize the full potential of their application. It is in this process of translation that the engineer must play a vital role. This talk will endeavor to describe the various problems, methods, and successes of the engineering scale-up process, with special emphasis on the scale-up of processes involving genetically-altered organisms. A historical perspective of scale-up will be presented, and future directions for research in this area will be suggested.

John H. Harrison, IV

## ABSTRACT of RESEARCH ACTIVITIES

Mechanism of Action of Dehydrogenase Enzymes

The overall objective of this laboratory been to attempt to understand the structure-function relationships in oligomeric enzymes. To that end the two forms of malate dehydrogenase from porcine heart have been used as a model system or vehicle for this research. Previous work by this laboratory has implicated the essentiality of four specific amino acid residues in the active center of the mitochondrial enzyme by means of selective chemical modifications. Similar studies by other laboratories has yielded information concerning two residues in the cytoplasmic enzyme. Recently, we have established the existence of both a pH and protein concentration dependent dissociation of the mitochondrial enzyme. Studies in which enzyme has been immobilized to an inert resin through a single linkage per dimer has allowed us to investigate the kinetic parameters of the enzymatically active monomeric form of this enzyme. Studies are presently continuing dealing with a further investigation of the structural conformations of the enzyme present during dissociation of the unbound enzyme and during stages of catalysis. Recently we have reported the observation that the metabolite citrate acts in a unique manner on the mitochondrial form of malate dehydrogenase. The overall effect is such as to enhance the enzymatic activity of the enzyme in the forward direction while suppressing the enzymatic activity in the back reaction. It has been proposed that this allosteric effect plays an important role in the control of metabolites and their passage into and out of the mitochondria. This investigation is presently being expanded inorder to more fully understand the role of this interation in the control of this enzyme in vivo as well as its correlation to the structure- function relationship that exists due to this interaction. In the future we plan to expand our investigations to attempt to understand the mechanism by which this protein, which is coded for in the mitochondria and synthesized in the cytoplasm, is transported into the mitochondria where it is biologically active. This investigation will include attempts to isolate the actual gene which is responsible for the production of this enzyme. Isolation of this material will enable us to carry out specific modification of amino acid residues at the primary level. This enzyme with the detailed information we have now collected acts as an excellent vehicle for attempts to understand not only protein-protein interaction but also the general mechanism of action of dehydrogenase enzymes.

# CHEMICAL MODIFICATIONS OF ENZYMES AS PROBES OF ACTIVE SITES AND MACROMOLECULAR STRUCTURE

H. Robert Horton

The catalytic activity of papain, a cysteine protease of plant origin, can be substantially increased by reaction with an active site-directed "affinity labeling" reagent, 2-chloromethyl-4-nitrophenyl (*N*-benzyloxycarbonyl)-glycinate. Structural analyses revealed that a specific tryptophyl residue (Trp-177), proximal to the essential thiol group (Cys-25) in the enzyme's three-dimensional structure, becomes alkylated through reaction with 2-hydroxy-5-nitrobenzyl chloride (HNB-Cl) which is generated *in situ* by enzymatic action. The resulting hydroxynitrobenzylated papain (HNB-papain) exhibits 3.5 times the catalytic activity of native papain in hydrolyzing benzoyl-L-arginine *p*-nitroanilide. Fluorescence measurements and mechanistic studies revealed that the covalently bound HNB "reporter group" increases the nucleophilicity of the catalytically essential Cys-25 sulfur atom, apparently by stabilizing the mercaptide-imidazolium ion pair at the active site (Cys-25, His-159), and thereby increases the rate of the initial nucleophilic attack by the enzyme on susceptible substrates. Proflavine, an acridine dye which can modify native papain's activity through reversible binding, also modifies the activity of HNB-papain. 2-Chloromethyl-4-nitrophenyl (*N*-benzyloxycarbonyl)glycinate can be applied to other cysteine proteases to probe their active sites for reactive tryptophyl residues.

Another form of chemical modification of enzymes involves immobilization through covalent bonding to insoluble matrixes. Such immobilization can be used to study the acquisition of tertiary structure in reductively denatured proteins through oxidation of their sulfhydryl groups to form disulfide bonds. Specific reversible chemical immobilization techniques have also been devised and applied to enzyme purification. By such means sulfhydryl oxidase has been successfully separated from γ-glutamyltransferase (two membrane-associated activities which had been thought to belong to a single enzyme).

# DESIGN AND POTENTIAL APPLICATION OF MODEL ENZYMES

by

Robert P. Bereman

Bioinorganic chemistry is a field which has developed over the past 10 years as an answer to the need to understand the role of metal sites in metalloproteins. The significance of the area is apparent when we realize that over one-third of all known proteins have a metal at the active site. An aspect of the area of bioinorganic chemistry that has been developed more recently is called "model system" chemistry. The initial concept was that small molecule complexes of metals were needed which mimiced aspects of the properties of the active site metal. These models are especially useful in providing the data base on which spectral properties of the protein can be interpreted. However, one seemingly significant aspect of model system chemistry hs been overlooked. The goal of the model system chemistry has been to design, test and evaluate complexes which mimic active sites of proteins. It is certainly clear that in some cases, these complexes might be extended to active form. That is, it is likely that artifical proteins can be developed in the near future.

A brief review of a few representative model systems will be presented. Some aspects of the work in our laboratories on copper (II) type I and type II sites will also be presented. Our work has recently been centered on attempts to duplicate the very unusual positive redox potentials of the electron transfer type I site and the unique clemical reactivity of type II sites. In addition we have been able to build active site systems onto supporting surfaces in order to ultimately utilize our models. A brief statement of near and distant future goals and prospects will be presented.

# Polymer Surface Control of the Biological Formation of Carbonates

Miles A. Crenshaw

A classical example of the induction of phase transformation by biopolymers is biological calcification. This process is most readily studied in molluscan shell formation because the crystals are relatively large, uniform, well-oriented and well-ordered. The polymeric fraction of the shell is formed into relatively large sheets, an arrangement that permits the study of its surface.

The polymeric sheets are formed prior to the deposition of calcium carbonate. The surface is characterized as being very hydrophobic with a hydrophobic index of 0.7 to 0.8. This hydrophobic surface is periodically interrupted by hydrophilic centers that bind calcium. These centers are the sites of initial crystal formation. These crystal grow rapidly along the C-axis, which is normal to the polymeric surface, until growth in this direction is inhibited. Horizontal crystal growth continues, at a lower rate, until the available surface is covered, resulting in an approximate hexagonal packing of crystals.

Gustavo P. Maroni
Department of Biology, UNC

Metallothionein in Drosophila

A number of eukaryotes have the ability to produce a low molecular weight protein, known as metallothionein (MT), which can bind group IIb divalent metal ions: $Zn^{++}$, $Cd^{++}$, $Hg^{++}$ and sometimes also copper and silver. Studies carried out in mammals have shown that the amount of metallothionein in tissues rises in response to $Cd^{++}$ and $Zn^{++}$.

Rat liver polysomes, from animals injected with zinc, are capable of directing in vitro translation of thionein, the apo-protein, at higher rates than polysomes from uninjected rats. It is apparent that this increase in the rate of synthesis is due to a concomitant increase in transcription of MT mRNA.

The structure of thionein is highly conserved among the species studied to date. It is a small protein (MW 6000 to 7000), very rich in cysteine and totally deficient in aromatic amino acids and histidine. In horse, humans and mouse there are two main forms of thionein; each has 61 amino acids, 20 of which are cysteine residues. Seven metal ions are bound by each molecule of protein in double or triple mercaptide complexes. Amino acid sequences indicate strong homologies; the positions occupied by the cysteines, as well as the serines and basic amino acids found in juxtaposition to them, are almost invariant. In addition to mammals, metallothionein has also been identified in birds, teleost fish, shellfish, Neurospora and yeast. In Neurospora, MT has been detected as a copper-binding protein; it is only 25 amino acids long and has remarkable homology to the N-terminal portion of mamalian MT, in particular in the location of its seven cysteines. For a general review see: Kojima, Y. and J.H.R. Kagi. Metallothionein. Trends in Biochemical Sciences 3:90-93 (1978). At least one of the mouse MT genes has been cloned and completely sequenced.

One of the roles ascribed to metallothionein is in Cd++ and perhaps Hg++ detoxification. It is also been suggested that thionein plays a role in the uptake and storage of Zn++ and $Cu^{+}$ in rats; thus securing the availability of these metals for cellular functions; but proof of a direct and necessary role by metallothionein in the supply of these and perhaps other trace elements is, I believe, missing. It is, in fact, one of the major challenges to research in this field to define more accurately the physiological role(s) of metallothionein. With this as one of our goals we set out to study metal-binding proteins in Drosophila. The ability to generate and analyze mutations in this organism would provide the means to study the physiological effects of certain specific deficiencies in these proteins.

We have purified a Drosophila protein with properties similar to those of MT: It binds cadmium, has low molecular weight (around 4,000), no aromatic amino acids and has cysteine. Its synthesis is induced by cadmium and copper but not by zinc. Using the mouse MT sequence as a probe we have detected two bands of hybridization to total, genomic Drosophila DNA digested with restriction enzymes and tested by the Southern blot procedure. We have also identified several lambda plaques of a cDNA bank capable of hybridizing to the same heterologous probe.

Adherence of Phytopathogenic Bacteria to Plant Cells. Ann G. Matthysse. Department of Biology, University of North Carolina, Chapel Hill, NC 27514.

The initial step in the induction of crown gall tumors by the gram negative bacterium Agrobacterium tumefaciens is the attachment of the bacteria to the plant host cells. Attachment is a multiple-step process. The first step involves the interaction of bacterial surface components, lipopolysaccharide and proteins, with surface components of the plant cell. This is followed by the elaboration of cellulose fibrils by the bacterium. These fibrils anchor the bacterium firmly to the host cell surface. Bacterial mutants which do not synthesize cellulose can be removed from wound sites on the surface of plants y water washing. Bacteria which do synthesize cellulose remain attached under these conditions. Agrobacterium produces some enzymes which degrade or alter polysaccharides on the host cell surface. These alterations may also play a role in the attachment of the bacterium to the plant cell.

# Interactions between marine fungi and wood borers

Jan Kohlmeyer

Studies on the nutrition of wood-boring molluscs and isopods usually consider the role of bacteria, whereas the ubiquitous lignicolous marine fungi are mostly neglected. Experiments with Teredo pedicellata carried out over 20 years ago demonstrated that the larvae accumulated preferably on wood that was "predigested" by fungi and settled on it, whereas fresh sterile wood was rejected (Kampf, Becker & Kohlmeyer 1959). Shipworm larvae need direct contact of the surface for chemotactic recognition, similar to settling larvae of Haliotis (Morse 1981). The origin of cellulase in adult shipworms appears to be unsolved, because one school of thought supports a bacterial origin (e.g. Rosenberg & Cutter 1973, Mann 1981), while others (e.g. Dean 1976) believe that microorganisms are not involved in the breakdown of the ingested wood. Fungi appear not to play a role in the digestion of wood by adult shipworms, but they are important in softening the wood surface and permitting penetration of the larvae before the shells are calcified.

Also in studies of Limnoriidae, emphasis was mostly on interactions between bacteria and the gribbles (e.g. Boyle & Mitchell 1981), and fungi were not considered. However, Kohlmeyer, Becker & Kampf (1959) have demonstrated that Limnoria tripunctata requires fungi in its diet for reproduction. The isopods ingest bacteria, but the digestive tract is free of bacteria which are digested (Boyle & Mitchell 1981). Intact fungal propagules and hyphae pass through the digestive tract (Kohlmeyer et al. 1959). Apparently, Limnoria does not produce a chitinase to dissolve the fungal cell walls, but may use the contents of damaged cells.

It is planned to study the interactions between fungi, shipworm larvae, and gribbles, respectively. Feeding experiments with wood borers, using fungi with radioactive tracers, will demonstrate the role of fungi in the settlement and nutrition of the borers, and, possibly, show new possibilities of wood protection in the sea.

# MATERIALS-RELATED SURFACE PHENOMENA

## Abstract

C. M. Balik and R. O. Scattergood
Department of Materials Engineering
North Carolina State University

Specific topics will be discussed that relate to surface phenomena in polymers, metals, and ceramics.

For polymers, these will include the interactions of polymers with surfaces, i.e. polymer adsorption and epitaxial crystallization processes. Both processes may be carried out from the melt or from solution, but discussion of the adsorption aspects will center around adsorption of polymers from solution. The effects of solution concentration, solvent, substrate surface energy, and time on the thickness of the adsorbed layer will be briefly presented. Epitaxial crystallization involves very specific interactions between polymer and substrate. These will be described, and a short review of polymer epitaxial systems studied and potential applications will be presented.

For metals and ceramics, surface interactions lead to a wide variety of important effects. In metals, chemical reactions at the surface, or the adsorption or absorption of impurity elements can have a profound influence on properties such as corrosion, fatigue and fracture, or synergistic combinations of these. With ceramics, which are normally poor conductors, local electric fields set up at the surface penetrate over appreciable depths. Semiconductor properties can be modified, and even such properties as fracture may be affected by these electrical perturbations.

# Immobilized Oxygen Carriers as Biomolecular Gas Extractors and Pumps

J. Bonaventura and C. Bonaventura
Marine Biomedical Center
Duke University Marine Laboratory
Beaufort, North Carolina 28516

## Abstract

A primary problem hindering the human species in its desire to explore and develop the oceans is a lack of readily breathable oxygen. Practical methods of underwater life support utilizing the vast amount of oxygen dissolved in ocean waters have not yet been developed. Fish and other water-breathing animals have, however, solved this problem. They do this by utilizing hemoglobin and other reversible oxygen-binding proteins as molecular oxygen pumps, transferring oxygen from the water-gill interface to the tissues where it is respired. Immobilized forms of oxygen carriers in flow-through reactors can be used in an analogous fashion. A method for extracting the dissolved oxygen from natural waters and other fluids based on immobilized oxygen carriers will be described.

Immobilized oxygen carriers may also be useful as blood substitutes, capable of reversible oxygen binding under physiological conditions. Carbon monoxide, nitric oxide, alkyl isocyanides can also be bound to immobilized hemoglobin and it may be possible to extract and "pump" these compounds as well. Techniques will be described which allow for immobilization of oxygen carriers at high concentrations in a state where they remain functional for long time periods. Specific requirements of an oxygen extraction system for underwater habitats will also be discussed. Other aspects of life support systems utilizing immobilized proteins and enzymes will be described to demonstrate the advantages of immobilized chemical species in hybrid biological/physical systems.

# The Design of Marine Structures

Stephen A. Wainwright
Zoology Department
Duke University

## Abstract

The major physical phenomenon that controls design features of structures in the ocean is drag. Reasons for and mechanisms of both the reduction and increase of drag, as they appear in marine organisms, will be presented. Structural mechanisms of marine organisms that allow either (1) bending without kinking, (2) bending without twisting, (3) twisting without bending or (4) shearing without bending will be discussed. One mechanism by which many marine animals change the mechanical properties of their structural materials will be shown. Robotic devices are important to our future. Most are inferior copies of human mechanisms: a plethora of more imaginative designs reside in marine organisms. We might profit from attention to designs that have had the advantage of 4 billion years of evolutionary selection.

B-20

# PROTEIN SYNTHESIS IN CHLOROPLASTS

Linda L. Spremulli
Associate Professor of Chemistry - UNC-CH

The mechanism of protein synthesis in bacteria such as E. coli is now fairly well understood and current experimental efforts are beginning to unravel the complexities of this process as it occurs in the cytoplasm of cells from high organisms. It is now well known that the chloroplasts of plant cells contain unique circular pieces of DNA and synthesize chloroplast specific messenger RNA and proteins. The synthesis of proteins within the chloroplast is carried out by a system which is distinct from the system found in the cell cytoplasm. But the relationship between the cytoplasmic and chloroplast protein synthesizing systems in the plant cell is not presently well understood. Some data suggests that, mechanistically, chloroplast protein synthesis is more closely related to that of bacteria than to that found in the cytoplasm of the plant cell. Other data indicates that the chloroplast system has features that distinguish it from both bacterial and cytoplasmic systems.

The focus of research in my laboratory is to characterize the protein synthesizing system of chloroplasts and to understand how protein synthesis within this organelle is regulated and how it is coordinated with protein synthesis in the cell cytoplasm. We are comparing the chloroplast translational system to those of E. coli, of the eukaryotic cytoplasm, of photosynthetic prokaryotes (the blue green algae), and of the chloroplasts of higher plants. We have begun by isolating the factors required for the elongation steps of polypeptide chain synthesis in chloroplasts of the unicellular photosynthetic eukaryote Euglena gracilis. Studies with these proteins indicate that the elongation factors from chloroplasts resemble those of bacteria more closely that those of the cytoplasm of the plant cell. But they differ from elongation factors of all other systems in size, antibiotic sensitivity and in immunological properties. Our initial studies on the initiation process for protein synthesis in Euglena chloroplasts indicates that it has a number of unique features which distinguish it from both bacterial and cytoplasmic protein synthesis, although, again, it appears to be overall more prokaryotic than eukaryotic in nature.

# Rational Design of Functional Proteins

P. Modrich, R. Webster, and T. Vanaman
Departments of Biochemistry and Microbiology/Immunology
Duke University Medical Center
Durham, N. C.

A large experimental and theoretical base currently exists which aims to explain elements of protein structure. Despite this substantial amount of information, the way in which protein linear sequence dictates patterns of folding, overall conformation and function is poorly understood. Technology now exists with which protein molecules can be systematically constructed and tested with regard to the above properties. To date, such studies have been approached only through analysis of naturally existing proteins and in a very limited number of cases, those synthesized by chemical methods. The latter has been applicable to relatively small moelcules (100 residues or less). We would like to consider here the application of contemporary techniques of molecular biology coupled with chemical and physical methods to address problems of protein folding and active site function and design. These possibilities will be explored using specific examples such as the intracellular $Ca^{2+}$-receptor calmodulin, and the enzymes it regulates.

# Using Concepts of Protein Topology and Structure to Design New Proteins

Jane S. Richardson
Department of Anatomy
Duke University

David C. Richardson
Department of Biochemistry
Duke University

## Abstract

We still have not mastered the general problem of predicting protein tertiary structure from the amino acid sequence, although there has been reasonable success recently with some of the simplest cases. It is tempting to hope, however, that our understanding might now be adequate for the easier inverse problem of specifying a sequences that would fold up into a given, simple tertiary structure. When working in the inverse direction one has the opportunity to load the dice (within the range allowed by all the other constraints) by choosing local sequence known to be very strong formers of turn, or helix, or whatever is desired in that position.

The only attempts of this sort made so far (by Gutte) have succeeded in producing activities at least similar to the ones desired, but the 3-dimensional structures apparently are not the ones intended. That is not too surprising, since knowledge of active site residues and secondary-structure predictions were used, but very little information about tertiary structures.

Our goal is to choose a small, simple pattern of tertiary structure idealized and extrapolated from the known examples, to design a sequence that should fold up to that pattern, and to test the process by synthesizing the amino acid sequence (being done by our collaborator, Bruce Erickson, at the Rockefeller) and determining its crystal structure. The design process takes into account as much as possible of the large body of descriptive, empirical generalizations about protein structure accumulated over the last 20 years by many people including ourselves. It also builds in features to simplify the synthesis and to allow later substitution of residues at one end to form an active site.

I will describe some of those design features and how one compromises among them, and indicate some features we wish to study further for incorporation into later attempts.

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## C. MICROECOLOGY WORKSHOP OCTOBER 18-19, 1982

### AGENDA

October 18

| | | |
|---|---|---|
| 8:00 a.m. | Biotechnology and North Carolina..... | Quentin Lindsey |
| 8:15 a.m. | Workshop Overview, Objectives, Logistics..... | Laura R. Meagher |
| 8:30 a.m. | Opening Comments..... | Joseph Bonaventura |
| 8:40 a.m. | Superoxide Dismutases: Exceptions to the Rules..... | Irwin Fridovich |
| 9:15 a.m. | Overview, Biotechnology..... | Darrell Stafford |
| 9:30 a.m. | Overview, Sensor and Detector Devices..... | Klaus Bachmann |

### SESSION I

### PERSPECTIVES ON MICROECOLOGY AND BIOTECHNOLOGY

| | | |
|---|---|---|
| 9:45 a.m. | Perspectives on Molecular Biology..... | Joseph Bonaventura, Chair |
| 11:00 a.m. | Perspectives on Unique Organisms..... | Fred Pfaender, Chair |
| 12:00 p.m. | LUNCH | |
| 1:00 p.m. | Perspectives on Products..... | Fred Pfaender, Chair |
| 2:00 p.m. | Overview, Microecology..... | Hans Paerl |
| 2:30 p.m. | Perspectives on Processes..... | Dirk Frankenberg, Chair |
| 3:30 p.m. | BREAK | |
| 3:45 p.m. | Perspectives on Processes, Continued..... | Dirk Frankenberg, Chair |
| 4:15 p.m. | Perspectives on Sensors..... | B. J. Copeland, Chair |
| 6:00 p.m. | DINNER | |
| 7:15 p.m. | Navy Interest in Microecology and Biotechnology | |
| 8:15 p.m. | Open Discussions and Social Hour | |

October 19

| | |
|---|---|
| 8:00 a.m. | Introduction to Purpose and Organization of Sessions II and III |
| 8:15 a.m. | Session II (Break-away group discussions) |
| 10:00 a.m. | BREAK |
| 10:15 a.m. | Conclusions of Each Group |
| 11:00 a.m. | Session III (Break-away group discussions) |
| 12:15 p.m. | LUNCH |
| 1:15 p.m. | Session III, Continued |
| 2:00 p.m. | Conclusions of Each Group |
| 2:45 p.m. | BREAK |
| 3:00 p.m. | Conclusions of the workshop (research recommendations); Overview Discussion of Workshop Conclusions led by Joseph Bonaventura, B. J. Copeland, Dirk Frankenberg, and Fred Pfaender. |
| 4:30 p.m. | Conference Ends |

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## D. CURRICULA VITARUM

Curricula Vitarum are available upon request from the North Carolina Biotechnology Center.